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Synthesis and Formation of T11223 and T1223 High-Tc Superconductors

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ABSTRAK

Kaedah penyediaan superkonduktor $Tl_2Ba_2Ca_2Cu_3O_{10}$ (Tl1223) dan $Tl_{0.5}Pb_{0.5}Sr_{1.6}Ca_{2.4}Cu_3O_9$ (Tl2223) menggunakan komposisi permulaan dan suhu sinter yang pelbagai dibincangkan. Tl2223 berfasa tunggal dengan T_{sifar} antara 106 dan 109 K telah disediakan menggunakan komposisi permulaan $Tl_2Ba_2Ca_2Cu_3O_{10}$ dan $Tl_{1.6}Ba_2Ca_{2.4}Cu_3O_{10}$ dalam sistem tidak tertutup dengan pemanasan sekitar 20 minit pada 910 °C dalam aliran O_2. Sampel berfasa utama Tl1223 (~ 70%) telah disediakan dengan komposisi $Tl_{0.5}Pb_{0.5}Sr_{1.6}Ca_{2.4}Cu_3O_9$ dengan memasukkan sampel dalam kerajang emas dan disinter dalam aliran oksigen pada 940°C selama 3 jam.

ABSTRACT

The preparation methods of $Tl_2Ba_2Ca_2Cu_3O_{10}$ (Tl1223) and $Tl_{0.5}Pb_{0.5}Sr_{1.6}Ca_{2.4}Cu_3O_9$ (Tl2223) superconductors using a variety of starting compositions and sintering temperatures are discussed. Single-phased Tl2223 with T_c between 106 to 109 K can be prepared using $Tl_2Ba_2Ca_2Cu_3O_{10}$ and $Tl_{1.6}Ba_2Ca_{2.4}Cu_3O_{10}$ as starting compositions in a non-closed system by heating around 20 min at 910°C in flowing O_2. Samples with Tl1223 major phase (~ 70%) can be prepared from $Tl_{0.5}Pb_{0.5}Sr_{1.6}Ca_{2.4}Cu_3O_9$ composition with the sample wrapped in gold foil and sintered in flowing oxygen at 940 °C for 3 hours.

Keywords : High-temperature superconductor, synthesis of: $Tl_2Ba_2Ca_2Cu_3O_{10}$ and $Tl_{0.5}Pb_{0.5}Sr_{1.6}Ca_{2.4}Cu_3O_9$, electrical resistance, X-ray diffraction

INTRODUCTION

The Tl-based high-temperature superconductors are an interesting family of compounds that can form phases with different numbers of CuO or TlO layers and critical temperatures (T_c). Single TlO layered $TlBa_2Ca_{n-1}Cu_nO_{2n+3}$ series and double TlO layered $Tl_2Ba_2Ca_{n-1}Cu_nO_{2n+4}$ series have been established with a maximum critical current T_c of 114 K (n=3) and 125 K (n=3) respectively. Preparation of Tl-based materials is more difficult compared to the well known

RE123 (RE-rare earth) materials due to the high volatility of Tl during heating and the growth of competing secondary phases (Abd-Shukor 1993). The Tl_2O_3 used in the preparation process is volatile at 717°C at atmospheric pressure and as such it is very volatile above that temperature. To minimize Tl_2O_3 loss, several groups used the precursor method where component oxide is mixed and calcined separately before Tl_2O_3 is added (Chen *et al.* 1994; Parkin *et al.* 1988; Sheng and Hermann 1988). Several methods also employ heating of samples in a closed system by using metallic foils (usually gold) to wrap the samples or sealed gold tubes (Luo *et al.* 1996; Subramanian *et al.* 1988). There are conflicting reports on preparation of Tl2223 using $Tl_2Ba_2Ca_2Cu_3O_{10}$ starting composition; some groups reported mixed Tl2223 and Tl2212 phases (Hazen *et al.* 1988; Parkin *et al.* 1988) while others reported dominant Tl2223 phase (Abd-Shukor and Hashim 1994; Xin *et al.* 1991). Bulk Tl1223 was repeatedly synthesized by partial substitution of Pb for Tl and using calcium- and copper-rich starting compositions (Liu *et al.* 1992; Paranthaman *et al.* 1994). Several groups have also reported the preparation of single phased Tl-based compounds using non-stoichiometric starting compositions (Liu *et al.* 1991; Nanjundaswamy *et al.* 1993; Parkin *et al.* 1988). Replacement of the exact amount of Tl_2O_3 that has evaporated during initial heating was also suggested (Abd-Shukor and Hashim 1994).

It is interesting to explore other synthesis methods of the Tl1223 and Tl2223 materials with the aim of producing better quality samples and shorter time for synthesis of the materials. Also, the use of gold foils/gold tubes which are expensive can be avoided if the Tl-based materials could be rapidly synthesized in a non-closed system. In this paper we report the synthesis of Tl2223 and Tl1223 in a non-closed system using different starting compositions. The effect of synthesizing Tl1223 in a closed system is also discussed. Powder X-ray diffraction and d. c. electrical resistance-temperature measurements are used to characterize their normal and superconducting properties.

EXPERIMENTAL DETAILS

The samples were prepared using the solid state synthesis method. Tl2223 was prepared from two different compositions: $Tl_2Ba_2Ca_2Cu_3O_{10}$ and $Tl_{1.6}Ba_2Ca_{2.4}Cu_3O_{10}$. Tl1223 was prepared from $Tl_{0.5}Pb_{0.5}Sr_{1.6}Ca_{2.4}Cu_3O_9$ and $Tl_{0.5}Pb_{0.5}Sr_2Ca_2Cu_3O_9$ compositions. Appropriate amounts of high purity (≥ 99.99 %) powders of Pb_2O_3, $SrCO_3$, BaO, CaO and CuO were mixed according to the relevant nominal composition and ground in an agate mortar. The powders were then calcined in air at around 900°C for 48 h with several intermittent grindings. Appropriate amounts of Tl_2O_3 were then added to the resultant powders according to the relevant nominal starting composition used. The powders were then mixed and pressed into pellets of 12.5 mm diameter and 3 mm thickness under a load of 5 tons using a hydraulic press. Some of the samples were wrapped in gold foil to minimize the loss of Tl during heating. The pellets were then placed in an alumina boat and sintered in a tube

furnace at different temperatures between 895°C and 920°C for Tl2223 and between 940°C and 955°C for Tl1223 in flowing oxygen for different durations followed by oven cooling to room temperature. The oxygen was kept flowing until the temperature reached below 300°C.

The samples were analyzed by powder X-ray diffraction method using Siemens D 5000 diffractometer with Cu-K_α radiation. The lattice parameter of the samples was obtained from the computer fittings of observed d values of diffractograms for individual samples. The volume percent of the 2223 and 2212 phases were estimated from the ratio of intensity of the low angle peaks ($2\theta \sim 5°$ for 2223 and $2\theta \sim 6°$ for 2212 i.e. the (002) reflection of both phases. The volume percent of the 1223 and 1212 phases were estimated from the ratio of intensity of the (110) peaks for both phases. Electrical resistance measurements between 35 K and 300 K were carried out using the four-point probe technique with silver paint contacts in conjunction with a closed cycle refrigerator from CTI Cryogenics Model 22.

RESULTS AND DISCUSSION

Table 1 shows the starting composition, sintering temperature, sintering time, resultant phases and T_c of the samples. All the XRD peaks are indexed with tetragonal unit cells. For $Tl_2Ba_2Ca_2Cu_3O_{10}$ composition, single-phased Tl2223 (sample 3A) with T_c of 106 K can be produced without wrapping in gold foil by fast heating at 910°C for 10 min followed by furnace cooling and heating it again for 10 min at the same temperature (10+10 min). The results also show that the optimum temperature for the formation of the 2223 phase is around 910°C and the short heating duration (~20 min) is sufficient for stabilization of the phase. The low T_c (84 K) for Sample 1A which was sintered for 300 min at 895°C indicates formation of a low temperature-phase. This may be caused by the loss of Tl due to the prolonged heating or the slightly lower sintering temperature used. For Sample 2A, sintering at 900°C for 10 min produces dominant Tl2223 (67%) but did not eliminate the minor 2212 phase (33%). XRD pattern for Sample 3A (*Fig. 1*) showed dominant 2223 phase with no significant impurity peaks. The calculated lattice parameters are a = 3.849 Å and c = 35.627 Å. The temperature dependent resistance measurements for Sample 3A and Sample 8C are shown in *Fig. 2*. Single-phased Tl2223 with T_c ~109 K can also be prepared from $Tl_{1.6}Ba_2Ca_{2.4}Cu_3O_{10}$ by heating around 894-900°C for 240 min (Sample 4B) or around 910°C for 20 min (Sample 6B). XRD pattern for Sample 4B and Sample 6B (figure not shown) showed no noticeable secondary or parasitic peaks which are sometimes expected when using non-stoichiometric starting compositions. Sintering at a higher temperature of 920°C (Sample 5B) causes increase in Tl2212 minor phase. Our observation on sample 3A is different from Y. Xin *et al.* (1991) who reported formation of 95% pure Tl2223 after heating at 895°C for 48 h. Our results indicate that 20 min of heating at 910°C is sufficient for the chemical reaction to produce 2223 phase. The shorter heating duration can also reduce excessive loss of thallium.

TABLE 1
The starting composition, sintering temperature, sintering duration, resultant phases and T_c of the samples. * indicates that sample was wrapped in a gold foil

Starting Composition	Sample	Sintering Temp. (°C)	Sintering Time (min.)	Resultant Phase Major	Minor	$T_{c\,zero}$ (K)	$T_{c\,onset}$ (K)
$Tl_2Ba_2Ca_2Cu_3O_{10}$	1A	895	300	unknown	unknown	106	84
	2A	900	10	2223 (67 %)	2212 (33 %)	123	111
	3A	910	10+10	2223(>98%)	-	123	106
$Tl_{1.6}Ba_2Ca_{2.4}Cu_3O_{10}$	4B	900	10				
		894	240	2223(>98%)	-	119	109
	5B	910	20				
		920	15	2223 (85 %)	2212 (15 %)	123	108
	6B	910	20	2223(>98%)	-	121	109
$Tl_{0.5}Pb_{0.5}Sr_{1.6}Ca_{2.4}Cu_3O_9$	7C	940	180				
		940	195	1212 (53 %)	1223 (47 %)	123	112
	8C*	940	180	1223 (70 %)	1212 (30 %)	122	112
	9C	940	180				
		950	180	1212 (63 %)	1223 (37 %)	125	112
	10C*	947	300	1212 (51 %)	1223 (49 %)	123	75
	11C*	940	180				
		950	180	1223 (56 %)	1212 (44 %)	125	112
	12C	940	360	1223 (52 %)	1212 (48 %)	120	112
	13C*	947	120	1212 (54 %)	1223 (46 %)	Not known	Not known
$Tl_{0.5}Pb_{0.5}Sr_2Ca_2Cu_3O_9$	14D	955	300	1212 (52%)	1223 (48%)	88	75
	15D*	940	300	1212 (55 %)	1223 (45 %)	Not known	Not known

Preparation of Tl1223 using $Tl_{0.5}Pb_{0.5}Sr_{1.6}Ca_{2.4}Cu_3O_9$ composition by sintering at 940°C for 3 h in O_2 with the sample wrapped in gold foil (Sample 8C) produces 1223 major phase with T_c of 112 K. The X-ray diffraction for Sample 8C (*Fig. 1*) showed 1223 major peaks (70%) with minor 1212 phase (30%). The calculated lattice parameters for the 1223 major phase are a = 3.820 Å and c = 15.18 Å. We also observed that sintering the $Tl_{0.5}Pb_{0.5}Sr_{1.6}Ca_{2.4}Cu_3O_9$ composition at 940°C in a non-closed system (without gold foil) (Sample 7C) or increasing the sintering temperature (Samples 9C,11C,13C) caused significant increase in Tl1212 phase. Preparation of Tl1223 using $Tl_{0.6}Pb_{0.5}Sr_2Ca_2Cu_3O_9$ composition by sintering in O_2 at 940 - 955°C for 6 h (Sample 14D and 15D) produced dominant Tl1212 phase together with minor Tl1223 phase. Comparing the resultant phases formed for Sample 8C and Sample 15D, it is clear that the formation of 1223 phase is promoted when the non-stoichiometric starting composition $Tl_{0.5}Pb_{0.5}Sr_{1.6}Ca_{2.4}Cu_3O_9$ is used. Our results also indicate that preparation of single phased Tl1223 may require heating in a closed system for longer than 3 h.

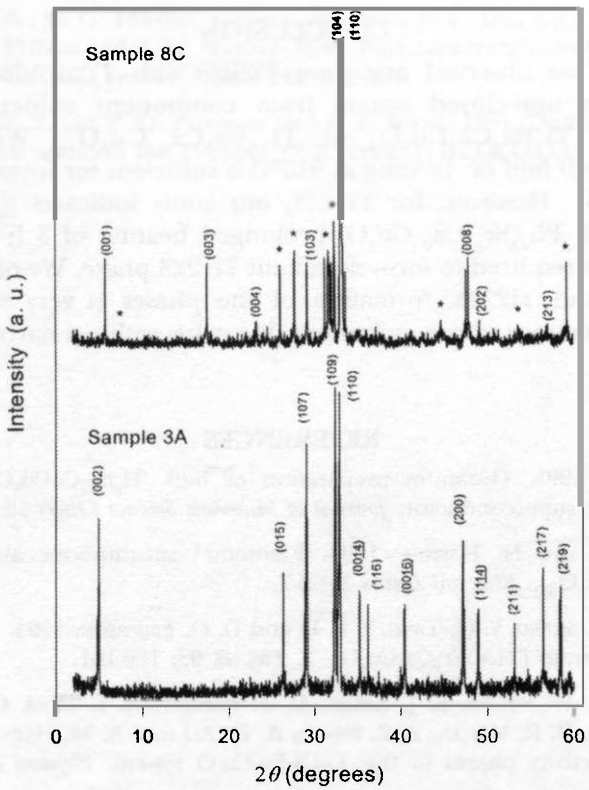

*Fig. 1: X-ray diffraction patterns for dominant Tl2223 (sample 3A) and Tl1223 (sample 8C). * indicates minor Tl1212 phase*

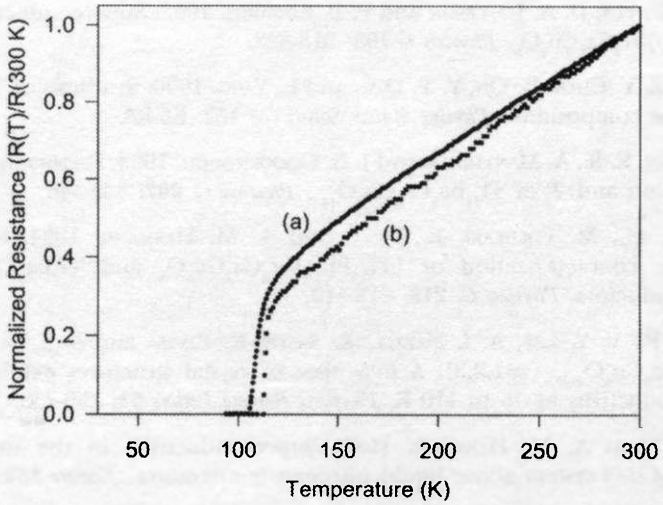

Fig. 2: Normalized resistance versus temperature curve for (a) Tl2223 (sample 3A) and (b) dominant Tl1223 (sample 8C)

CONCLUSION

In conclusion, we observed that pure Tl2223 with T_c of 106-109 K can be prepared in a non-closed system from component oxides with starting compositions $Tl_2Ba_2Ca_2Cu_3O_{10}$ or $Tl_{1.6}Ba_2Ca_{2.4}Cu_3O_{10}$. We found that approximately 20 min of heating at 910°C is sufficient for formation of single-phased Tl2223. However, for Tl1223, our study indicates that for starting composition $Tl_{0.5}Pb_{0.5}Sr_{1.6}Ca_{2.4}Cu_3O_9$ prolonged heating of 3 h or longer in a closed system is required to form dominant Tl1223 phase. We observed that for both Tl1223 and Tl2223, formation of the phases is very sensitive to the sintering temperature whose optimum value exists within a narrow temperature range.

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Spektroskopi Impedans Getah Asli Terepoksida (SMR5 dan ENR25)

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ABSTRAK

Kertas ini melaporkan pengukuran spektroskopi impedans getah asli terepoksida yang dilakukan dengan menggunakan spektrometer dielektrik pada suhu 303, 313, 333, 353 dan 373 K dalam julat frekuensi 10^{-2} ke 10^{6} Hz. Hasil eksperimen menunjukkan bahawa frekuensi santaian atau cirian bagi Z'', komponen maya impedans, bertambah dengan penambahan suhu. Model rangkaian litar setara (RC) yang dicadangkan di dalam kajian ini adalah bersetuju dengan data uji kaji yang diperoleh untuk julat frekuensi 10^{-2} - 10^{6} Hz.

ABSTRACT

This paper reports the measurement of impedance spectroscopy of epoxydized natural rubber by using a dielectric spectrometer at temperatures of 303, 313, 333, 353 and 373 K in the frequency range of 10^{-2} to 10^{6} HZ. Experimental results showed that the characteristic or relaxation frequency of Z'', the imaginary component of the impedance, increased with increasing temperature. The equivalent circuit network model (RC) proposed in this work is in good agreement with the experimental data obtained for the frequency range of 10^{-2} - 10^{6} Hz.

Kata kunci : Impedans, masa santaian, litar setara

PENGENALAN

Spektroskopi impedans banyak digunakan terutama untuk menganalisis santaian elektrik pada bahan ionik (Zhigao *et al.* 1992) dan kaca pengalir (Wong dan Angell 1976; Macedo *et al.* 1972). Oleh itu pencirian impedans dalam getah asli terepoksida dapat diperihalkan daripada pengukuran impedans spektroskopi menggunakan sumber ulang alik yang dikenakan kepada sampel berkenaan.

Bila suatu medan ulang alik dengan keupayaan $V^*(\omega)=V_0 \exp j(\omega t)$ merentasi suatu litar RC selari, arus ulang alik $I^*(\omega)=I_0 \exp j(\omega t+\theta)$ akan teraruh dalam sistem tersebut, iaitu V_0 dan I_0 merupakan amplitud bagi voltan dan arus, manakala j dan ω adalah masing-masing nombor kompleks (j^2 = -1) dan frekuensi sudut medan ulang alik serta θ adalah beza fasa (Hippel 1954). Oleh itu impedans bagi sistem adalah

$$Z^*(\omega) = \frac{V(\omega)}{I(\omega)} = Z'(\omega) - jZ''(\omega) \quad (1)$$

Kaitan impedans kompleks dengan kapasitan boleh ditulis sebagai berikut:

$$Z^*(\omega) = \frac{1}{j\omega C^*(\omega)} \quad (2)$$

dengan $C^*(\omega) = C'(\omega) - jC''(\omega)$, iaitu C' adalah bahagian nyata dan C'' adalah bahagian khayal yang mana $C'(\omega)$ adalah nilai pengukuran kapasitan nyata (C) terhadap frekuensi dan $C''(\omega)$ dikira dari nilai konduktans (G) terhadap frekuensi ($C''(\omega) = G/\omega$). Oleh itu

$$Z^*(\omega) = \frac{C^*(\omega)}{\omega\left(C'(\omega) + C''^2(\omega)\right)} - j\frac{C'(\omega)}{\omega\left(C'^2(\omega) + C''^2(\omega)\right)} \quad (3)$$

dengan membandingkan (1) dan (3), bahagian nyata dan khayal impedans untuk kes ini boleh ditentukan.

Bagi litar setara RC selari (*Rajah 1a*), impedans kompleks pada frekuensi sudut ialah

$$Z^*(\omega) = \frac{R}{1+(\omega\tau)^2} - j\frac{R\omega\tau}{1+(\omega\tau)^2} \quad (4)$$

dengan $\tau = RC$ ialah masa santaian. Oleh kerana impedans yang diperoleh melalui hubungan (4) adalah lebih sesuai untuk sampel seperti cecair elektrolit (kes unggul), selanjutnya perlu mengubah suai formula empirik untuk menyesuaikan kes-kes uji kaji yang tidak unggul khususnya sampel pepejal. Oleh itu, kapasitor semesta yang diperkenalkan oleh Jonscher (Jonscher 1983) perlu digunakan iaitu

$$C^*(\omega) = B(j\omega)^{p-1} = C_0\left(\frac{f}{f_0}\right)\left[sin\left(p\frac{\pi}{2}\right) - jkos\left(p\frac{\pi}{2}\right)\right] \quad (5)$$

dengan B ditakrifkan sebagai $C_0\left(\frac{1}{\omega_c}\right)^{p-1}$ dan $\omega_c = 2\pi f_c$, dengan menggunakan kapasitor semester, pemerihalan spektroskopi impedans bagi sistem ini akan menjadi lebih mudah.

Bagi menyesuaikan hasil uji kaji dengan pengiraan formula empirik, p mengambil nilai di antara 0 dan 1. $C^*(\omega)$ sebenarnya adalah gabungan selari antara kapasitor $C(\omega) = b\omega^{p-1}$ dan konduktans $G(\omega) = a\omega^p$ (4). Jika $p = 1$, ianya adalah kapasitor dan bila $p = 0$, ianya adalah impedans. Walau bagaimanapun rangkaian litar setara boleh dibentuk berdasarkan gabungan selari atau siri kapasitor semesta (*Rajah 1b*) yang bersiri dengan rintangan elektrod, R_s, impedans boleh dinyatakan sebagai

$$Z^*(\omega) = \frac{1}{j\omega\left(C_1^* + C_2^*\right)} + R_s$$

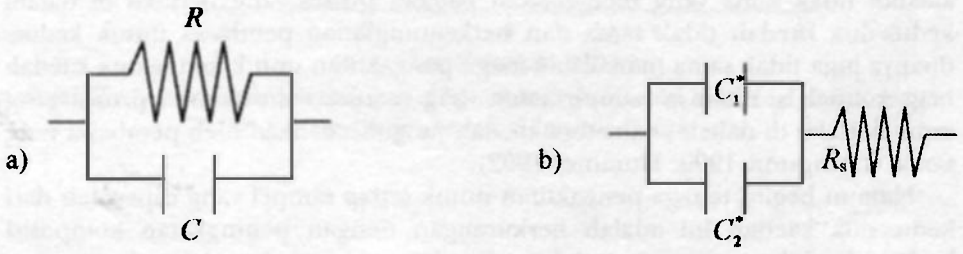
$$= \frac{R_0}{\left(\frac{f}{f_c}\right)^p kos\left(p\frac{\pi}{2}\right) + \left(\frac{f}{f_c}\right)^q kos\left(q\frac{\pi}{2}\right) + j\left[\left(p\frac{\pi}{2}\right) + \left(\frac{f}{f_c}\right)^q sin\left(q\frac{\pi}{2}\right)\right]} + R_s \qquad (6)$$

dengan $C_1^* = A(j\omega)^{p-1}$ dan $C_2^* = B(j\omega)^{q-1}$ (12), manakala A dan B ditakrifkan sebagai $A = \frac{1}{R_0}\left(\frac{1}{\omega_c}\right)^{p-1}$ dan $B = \frac{1}{R_0}\left(\frac{1}{\omega_c}\right)^{q-1}$ dengan $\omega_c = 2\pi f_c$. Pemalar p dan q mengambil nilai di antara 0 dan 1.

Selain penyerakan terhadap frekuensi, impedans kompleks juga berubah dengan suhu. Oleh itu tenaga pengaktifan boleh diperoleh dari kecerunan plot Arrhenius melalui hubungan (7) iaitu (Jonscher 1977):

$$f_c = f_0 exp\left(-\frac{E}{kT}\right) \qquad (7)$$

dengan f_c adalah frekuensi apabila magnitud impedans khayalan maksimun, f_0 merupakan frekuensi pada suhu $T^1 = 0$, E, k dan T masing-masing merupakan tenaga pengaktifan, pemalar Boltzman dan suhu dalam unit Kelvin.



Rajah 1 : (a) Kombinasi RC secara selari (b) Kombinasi kapasitor secara selari

KAEDAH UJI KAJI

Dua jenis sampel getah asli terepoksida iaitu SMR5 dan ENR25 dengan setiap satunya mengandungi komposisi karbon 50 dan 75 peratus, sama seperti yang dilaporkan oleh Mohd Noor (Mohd Noor *et al.* 1999), telah digunakan untuk kajian ini. Sampel-sampel ini kemudiannya dirujuk sebagai a_1 dan a_2 untuk SMR5 (50% karbon) dan SMR5 (75% karbon) serta b_1 dan b_2 untuk ENR25 (50% karbon) dan ENR25 (75% karbon). Penyediaan sampel untuk kegunaan kajian ini divulkankan pada suhu 423 K di dalam satu bekas besi yang dibuat khusus bersaiz dalaman 17.6 cm x 17.6 cm dengan ketebalan 0.05, 0.1 dan 0.2 cm dengan masa pemvulkanan masing-masing 10, 7, 10 dan 7 minit untuk setiap satu.

Impedans kompleks sampel-sampel ini dapat ditentukan dari pengukuran kapasitans kompleks melalui persamaan (3). Bagi tujuan ini, sampel-sampel berkenaan diletakkan di antara dua elektrod dalam sel yang boleh dikawal suhunya. Pengukuran kapasitans kompleks sampel-sampel ini dilakukan dengan teknik piawai (Mohd Noor *et al.* 2001) menggunakan Spektrometer Dielektrik dalam julat frekuensi 10^2 ke 10^6 Hz pada suhu 303, 313, 333, 353 dan 373 K.

HASIL DAN PERBINCANGAN

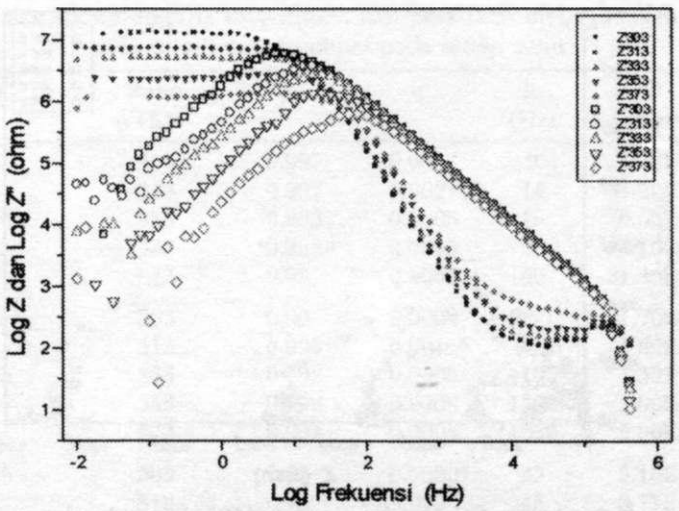
Rajah 2 menunjukkan penyerakan impedans iaitu log impedans kompleks lawan log frekuensi untuk setiap suhu. Hasil penyerakan ini, maklumat masa santaian f_c dalam julat frekuensi (jenis proses pengutuban) dikenal pasti. *Rajah 2* juga menunjukkan bahawa nilai frekuensi puncak setiap penyerakan impedans khayal sampel a_1, teranjak kekanan (meningkat) iaitu meningkat dengan peningkatan suhu. Plot Arrhenius frekuensi puncak f_c lawan T^1 (Jonscher 1977) untuk sampel ini ditunjukkan dalam *Rajah 3*.

Tenaga pengaktifan untuk semua sampel a_1, a_2, b_1 dan b_2 didapati masing-masing bernilai 0.25 ± 0.03 eV, 0.12 ± 0.03 eV, 0.35 ± 0.02 eV dan 0.20 ± 0.08 eV. Penganalisisan berdasarkan kekonduksian arus terus di dalam sampel-sampel ini mendapati tenaga pengaktifan untuk sampel-sampel a_1, a_2, b_1 dan b_2 masing-masing bernilai 0.31 ± 0.03 eV, 0.17 ± 0.04 eV, 0.34 ± 0.03 eV dan 0.27 ± 0.08 eV (Mohd Noor *et al.* 2000). Perbandingan di antara kedua-dua nilai ini didapati bahawa tenaga pengaktifan yang diperoleh melalui kedua-dua kaedah tidak sama yang menjelaskan bahawa proses yang berlaku di dalam kedua-dua kaedah tidak sama dan berkemungkinan pembawa untuk kedua-duanya juga tidak sama manakala tenaga pengaktifan untuk kedua-dua kaedah bagi sampel b_1 nilainya hampir sama yang menjelaskan bahawa proses yang sama berlaku di dalam kedua-dua kaedah yang disebabkan oleh pembawa yang sama (Livingston 1999; Hummel 1992).

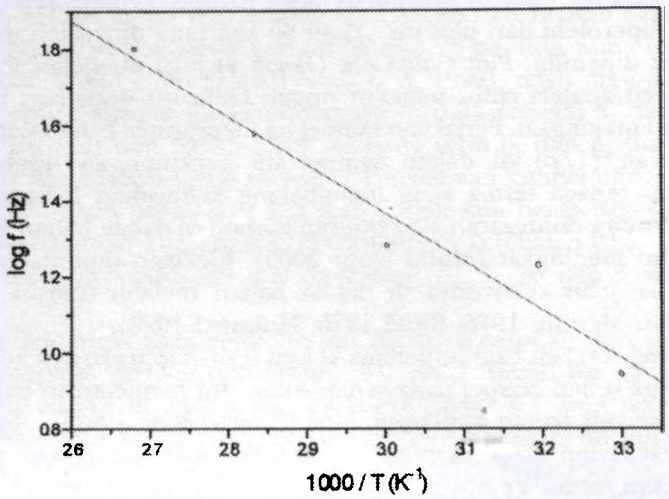
Namun begitu tenaga pengaktifan untuk setiap sampel yang diperoleh dari kedua-dua kaedah ini adalah berkurangan dengan peningkatan komposisi karbon. Penjelasan tentang perlakuan ini akan dibincangkan dalam kertas yang berasingan.

Plot Cole-Cole pula ditunjukkan dalam *Rajah 4*. Pada asasnya diharapkan lengkung separuh bulatan sempurna atau hampir kepada separuh bulatan sempurna diperolehi dari plot ini. Hasil uji kaji yang diperoleh menunjukkan harapan ini dipenuhi. Plot Cole-Cole (*Rajah 4*) juga menjelaskan bahawa Z0 semakin kecil apabila suhu semakin tinggi. Oleh itu impedans berkurangan apabila suhu meningkat. Perlakuan sampel ini menyerupai bahan semikonduktor. Kekonduksian $(1/\rho)$ di dalam sampel ini berkurangan disebabkan oleh peningkatan tenaga terma yang menghalang kelincahan lompatan elektron iaitu jarak antara zarah-zarah atau butiran karbon di dalam bahan semakin jauh apabila suhu meningkat (Mohd Noor 2000). Elektron dipindahkan daripada satu jalur ke jalur seterusnya di dalam bahan melalui transisi antara jalur (Ashroft dan Mermin 1976; Kittel 1976; Hummel 1992).

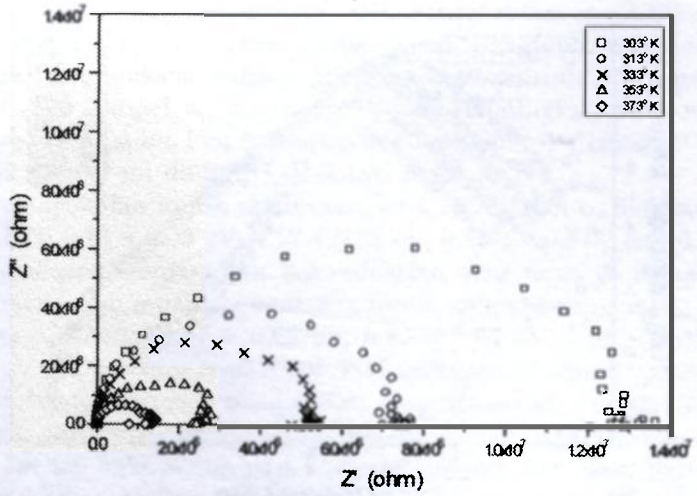
Lengkung serakan bagi impedans khayal terhadap frekuensi adalah bersifat simetri untuk setiap sampel pada setiap suhu. Ini menjelaskan bahawa sampel ini adalah sejenis bahan semikonduktor (Zhigao *et al.* 1992). *Rajah 5* adalah contoh kesesuaian data eksperimen dengan data pengiraan yang dibuat menggunakan formula empirik impedans kompleks untuk sampel a_1 pada suhu 373 K. Nilai-nilai dari formula empirik yang bersesuaian dengan data kajian disenaraikan dalam Jadual 1.



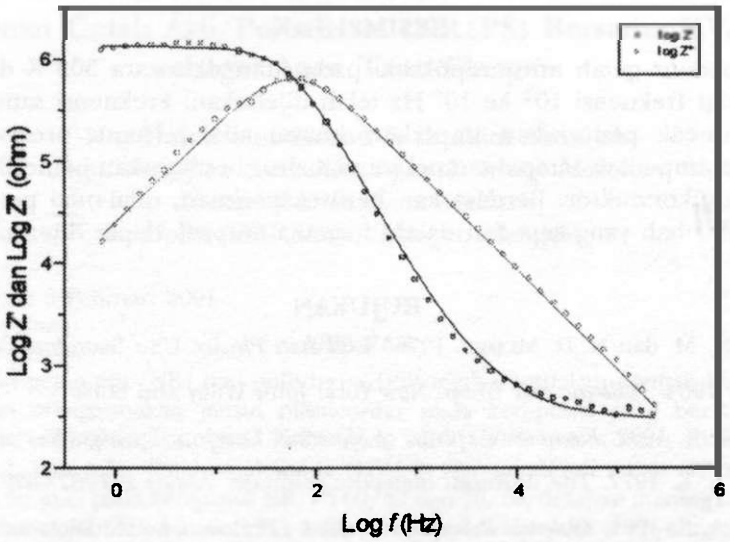
Rajah 2 : Log impedans kompleks lawan log frekuensi pada setiap suhu bagi sampel SMR5 dengan kandungan karbon 50 peratus (sampel a_1)



Rajah 3: Plot frekuensi puncak melawan T_1 bagi sampel SMR5 dengan kandungan karbon 50 peratus (sampel a_1)



Rajah 4 : Plot impedans Cole-Cole bagi sampel SMR5 dengan kandungan karbon 50 peratus pada suhu 303, 313, 333, 353 dan 373 K



Rajah 5 : Contoh kesesuaian hasil uji kaji dan formula empirik bagi sampel SMR5 dengan 50 peratus komposisi karbon pada suhu 373 K. Simbol adalah data eksperimen manakala garis adalah berdasarkan perkiraan dari litar setara

JADUAL 1
Hasil kesesuaian secara empirik eksperimen dan perkiraan menggunakan persamaan (6) untuk setiap sampel pada setiap suhu

Sampel	Suhu (K)	p	q	fc (Hz)	R0 (ohm)	Rs (ohm)
SMR5	303	0.997	0.00	9	1.28E+7	170
50%	313	0.997	0.002	14	7.50E+6	170
karbon	333	0.993	0.0005	19	6.50E+6	170
	353	0.993	0.0005	38	3.10E+6	170
	373	0.98	0.0005	60	1.45E+6	170
SMR5	303	0.99	0.0004	850	1.70E+5	45
75%	313	0.993	0.0015	850	1.80E+5	45
karbon	333	0.992	0.0005	1100	1.30E+5	45
	353	0.992	0.0009	1300	1.00E+5	45
	373	0.991	0.0005	1650	5.00E+5	45
ENR25	303	0.95	0.00001	35	5.10E+5	130
50%	313	0.95	0.005	45	3.77E+5	130
karbon	333	0.90	0.02	80	1.99E+5	130
	353	0.86	0.04	170	9.00E+4	130
	373	0.80	0.16	350	4.40E+4	130
ENR25	303	0.98	0.00001	250	2.20E+5	80
75%	313	0.987	0.0004	440	1.00E+5	80
karbon	333	0.984	0.00003	450	9.70E+4	80
	353	0.98	0.002	500	8.30E+4	80
	373	0.98	0.006	1430	1.50E+4	80

KESIMPULAN

Sifat impedans getah asli terepoksida pada suhu di antara 303 K dan 373 K dalam julat frekuensi 10^{-2} ke 10^{6} Hz telah dijelaskan. Frekuensi santaian atau cirian puncak penyerakan impedans khayal adalah suatu proses teraktif. Perlakuan impedans sampel-sampel ini dianalisis berdasarkan pemerihalan sifat bahan semikonduktor. Berdasarkan hasil eksperimen, nilai-nilai pemalar dan pemboleh ubah yang sepadan dalam formula empirik dapat ditentukan.

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Adunan Getah Asli/Polistirena (NR/PS) Bersama HVA-2 sebagai Agen Taut Silangan

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ABSTRAK

Adunan getah asli (NR) dan polistirena (PS) telah disediakan dengan kaedah leburan menggunakan mesin plasticorder pada komposisi yang berlainan. Adunan telah dilakukan pada kadar rpm, suhu 120°C dalam masa 15 minit. Penambahan *m*-fenilenabismaleimida (HVA-2) dan getah asli cecair (LNR) ke dalam adunan pada komposisi NR/PS 60/40 dan 70/30, didapati meningkatkan sifat-sifat mekanik adunan. HVA-2 dipercayai berkeupayaan menyeragamkan pembentukan taut silangan terutamanya dalam fasa getah. Komposisi optimum HVA-2 dan LNR bagi adunan NR/PS 60/40 adalah masing-masing 1.2 – 1.3 phr dan 5% LNR. Bagi adunan 70/30, kepekatan optimum adalah 1.6 – 1.7 phr dan 10% LNR.

ABSTRACT

Polystyrene (PS) and Natural Rubber (NR) were prepared by melt blending of the materials in a plasticorder mixer for two compositions. The processing conditions were a mixing rate of 50rpm, a duration of 15 min and a temperature of 120°C. The mechanical properties of the blend had improved significantly with the addition of HVA-2 and LNR into the blends. The effect of *m*-phenylenebismaleimide (HVA-2) and Liquid Natural Rubber (LNR) on the curing properties of NR/PS 60/40 and 70/30 blends were evaluated. Formation of cross links within the phase was observed to increase on additionof HVA-a. The crosslinking reactions seemed to focus predominantly in the NR phase as these reagents were more soluble in it. The most efficient composition of HVA-2 were of 1.2 – 1.3 phr for blends of NR/PS 60/40 with 5% LNR. At a composition of NR/PS 70/30 with 10% LNR, the optimum amount of HVA-2 as 1.6 – 1.7 phr.

Kata kunci: Polistirena, getah asli, HVA-2, getah asli cecair

PENDAHULUAN

Pengadunan dua atau lebih polimer yang berlainan boleh meningkatkan sebahagian daripada sifat-sifat fizikal dan mekanikal. Secara umum getah asli termoplastik (TPNR) boleh dibahagi kepada dua kategori iaitu TPNR bergred lembut dan TPNR bergred separa tegar (Tinker 1987: Gelling dan Tinker 1988). Umumnya jika komposisi termoplastik melebihi NR, TPNR adalah sebagai separa tegar yang mempunyai kekuatan hentaman yang tinggi terutama pada suhu rendah (Gelling dan Tinker 1988). Bagi TPNR bergred lembut pula,

kandungan NR adalah tinggi dan boleh dikelaskan sebagai elastomer termoplastik.

Penambahan HVA-2 ke dalam adunan NR/PP boleh menambah kekuatan tegangan dan kekuatan rintangan koyakan adunan (Elliott dan Tinker 1985). Penambahan HVA-2 ke dalam adunan getah/termoplastik didapati boleh menyempurnakan lagi beberapa sifat adunan. Ini telah ditunjukkan oleh beberapa penyelidik terhadap adunan NR/PP (Tinker 1984; Campbell 1978; Methew dan Tinker 1986). Menurut Tinker (1984) sifat hentaman meningkat dengan penambahan sedikit HVA-2 yang bertindak sebagai agen taut silang dalam fasa getah. Dijangkakan HVA-2 dapat bertindak sebagai agen pematangan kepada adunan NR/PS. Artikel ini akan membincangkan penyediaan TPNR, adunan NR/PS dengan *m*-fenilenabismaleimida (HVA-2) sebagai agen pematangan. Secara ringkas mekanisme pemvulkanan menggunakan HVA-2 ditunjukkan seperti berikut (Kovacic dan Hein 1959):

POOP	→	2PO•
PO• + RH	→	POH + R•
R• + MM	→	RMM•
RMM• +RH	→	RMHM +R•
R• + RMHM	→	RMHMR•
RMHMR•+RH	→	RMHMHR + R•
R• + R•	→	R – H
R• + RMM •	→	RRMM
R• + RMHMR•	→	RMHMRR
RMM•1 + RMM•	→	RMMMMR
RMHRM• + RMM•	→	RMHRMMMR
RMHRM• + RMHRM•	→	RMHRMMRHMR

Iaitu POOP ialah organik peroksida dan MM ialah bismaleimida.

EKSPERIMEN

Bahan-bahan

Bahan-bahan yang digunakan ialah getah asli (NR) jenis SMR-L yang dibekalkan oleh Guhrie (M) Sdn Bhd., Polistirena (PS) diperoleh dari Petrochemical (M) Sdn Bhd., Toluena yang berjenama Merck dari Germandan agen penaut silang *m*-feilenabismaleimida (HVA-2) didapati dari Trade TCI Mark, Tokyo.

Penyediaan Sampel

Getah asli cecair (LNR) dihasilkan melalui tindak balas pengoksidaan fotokimia getah asli (Ibrahim 1996). Adunan NR/PS disediakan dalam 2 komposisi iaitu 70/30 dan 60/40 menggunakan HVA-2 sebagai agen penaut silang bersama LNR sebagai pengserasi. Pengadunan dilakukan selama 15 minit pada kadar putaran rotor 50 rpm dan suhu 120°C. Mesin pengadunan yang digunakan

adalah Brabender PL 2000 berkawalan komputer dengan pencampur W50E/2 yang berisi padu 55 cm^3.

NR dimasukkan ke dalam mesin pengadun dan diikuti satu minit kemudian oleh LNR. PS dimasukkan pada minit ke-3 dan agen penaut silang pada minit ke-5, bahan diadunkan selama 15 minit. Adunan yang masih panas dikeluarkan dan ditekan di dalam penekan panas hidrolik. Tekanan sebanyak 7 tan metrik dikenakan selama 10 minit pada suhu 170°C. Kepingan setebal 1 mm dan 3 mm yang terhasil kemudian dikeluarkan dan dibiarkan matang selama 3 hari sebelum pengujian fizikal dilakukan.

Kandungan HVA-2 yang digunakan pada tahap 0.0, 0.1, 0.9, 1.7, 2.5 phr. Adunan dilakukan mengikut formulasi seperti di dalam Jadual 1.

JADUAL 1
Formulasi adunan

	Komposisi 60:40			Komposisi 70:30		
	I	II	III	I	II	III
NR (%)	60	55	53	70	65	60
PS (%)	40	40	40	30	30	30
LNR(%)	0	5	7	0	5	10
HVA-2 (phr)	x	x	x	x	x	x

Petunjuk : x adalah kuantiti HVA-2 yang berubah

PENCIRIAN SIFAT MEKANIK

(a) Ujian Regangan

Mesin ujian Universal Instrom Model 4301 dengan sel muatan sebesar 1 kN digunakan. Kepingan sampel setebal 1 mm dipotong kepada bentuk "dumbel", untuk ujian tegangan. Kelajuan tarikan ialah 50 mm/minit mengikut standard ASTM D 638. Suhu dan kelembapan makmal semasa ujian dijalankan adalah T = 25°C dan RH = 65%. Ujian dijalankan ke atas 7 sampel dan nilai purata diambil dari 5 bacaan terbaik.

(b) Ujian Kekerasan

Kekerasan diukur denan menggunakan Durometer Shore A dari jenis Zwick Model 3100 berasaskan ASTM D-2240-. Tebal sampel yang diperlukan tidak kurang dari 3 mm dan ukuran dibuat berdasarkan kepada kedalaman penembusan indentor tersebut.

(c) Ujian Hentaman

Ujian ini dijalankan mengikut kaedah Izod menggunakan mesin Bandul Digital Universal Froctoscope (CEAST) 6546/000 berdasarkan kepada ASTM D 256-88. Sampel ujian dari kepingan 3 mm dipotong menggunakan alat Hollow Die

Puncher dan pemotong Notch Davenport digunakan untuk membentuk takuk 1 mm pada bahagian tengah.

Darjah Taut Silang

Ujian pembengkakan dijalankan mengikut piawaian ASTM D 3616. Tiga keping sampel ujian yang berukuran 1 cm x 1 cm ditimbang dan dimasukkan ke dalam tabung uji yang mengandungi pelarut toluena selama 24 jam. Kemudian sampel dikeluarkan dan dilapkan permukaannya dengan kertas turas lalu ditimbang dengan segera. Sampel tersebut dikeringkan di udara selama satu hari sebelum dikeringkan di dalam ketuhar vakum pada suhu 60°C sehingga mendapat berat malar.

KEPUTUSAN DAN PERBINCANGAN

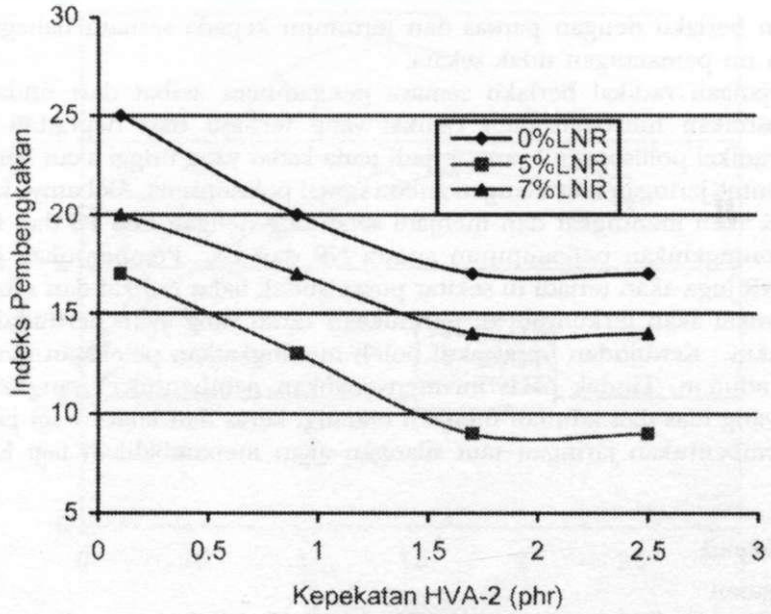
Indeks Pembengkakan

Rajah 1(a) dan *Rajah 1(b)* masing-masing menunjukkan pengaruh HVA-2 terhadap pembentukan taut silangan bagi adunan 60/40 dan adunan 70/30. HVA-2 berkesan dalam mewujudkan interaksi di antara fasa dalam adunan. Perubahan dalam indeks pembengkakan dengan komposisi HVA-2 menunjukkan wujudnya rangkaian taut silangan dalam matriks apabila reagen ini dicampur ke dalam adunan.

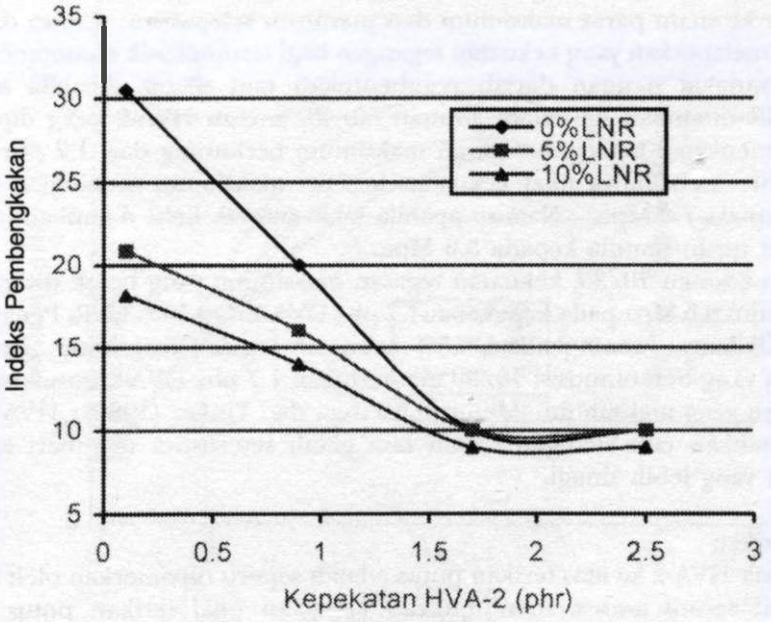
Rajah 1(a) menunjukkan bahawa darjah taut silangan bagi adunan 0%, 5% dan 7% LNR meningkat dengan penambahan HVA-2 dalam adunan sehingga 1.7 phr dan seterusnya menjadi malar. Bagi adunan 7% LNR, darjah taut silangan rendah apabila dibandingkan dengan adunan 5% LNR tetapi lebih tinggi daripada adunan 0% LNR. HVA-2 telahpun dilapor dapat bertindak sebagai agen taut silang dalam fasa getah (Methew dan Rinker 1986). Kehadiran LNR sebanyak 5% dalam adunan boleh mengurangkan indeks pembengkakan sebanyak 47% manakala apabila lebih banyak LNR ditambah iaitu 7%, indeks pembengkakan hanya berkurang sebanyak 18% pada kepekatan 1.7 phr HVA-2. Nilai indeks pembengkakan berkurang dari 25 kepada 17 apabila adunan ditambah 5% LNR pada kepekatan 0.1 phr HVA-2. LNR sebagai pengserasi dalam adunan TPNR telapun dilaporkan (Sahrim dan Ibrahim 1992; Sahrim *et al.* 1994). *Rajah 1(b)* menunjukkan bahawa pada kepekatan 1.7 phr HVA-2 nilai indeks pembengkakan bagi ketiga-tiga adunan adalah hampir sama dan seterusnya menjadi malar. Pada kepekatan 0.1 phr HVA-2 nilai indeks berkurang masing-masing dari 30.5 kepada 18.2 dna 20.9 apabila adunan ditambah 10% dan 5% LNR.

Ini akan menghasilkan adunan berkekuatan tinggi dan akan terjadi apabila kuantiti penjana radikal dan tahap ricihan dapat diimbangkan.

Degradisi bahan berlaku semasa proses percampuran adunan. Daya ricihan dapat memutuskan jaringan taut silangan dan meruntuhkan morfologi yang dibina. Pemprosesan berlebihan akan menghasilkan adunan yang lembut dengan kekuatan regangan yang rendah. Keadaan ini terjadi apabila pembentukan taut



(a) Adunan 60/40



(b) Adunan 70/30

Rajah 1: Pengaruh HVA-2 terhadap indeks pembengkakan adunan NR/PS

silangan berlaku dengan pantas dan tertumpu kepada sesuatu bahagian dan dengan itu pematangan tidak sekata.

Penjanaan radikal berlaku semasa pengadunan akibat dari tindak balas pengabstrakan hidrogen oleh radikal yang terhasil dari degradasi HVA-2. Makroradikal poliisoprenil yang terjadi pada kadar yang tinggi akan bercantum membentuk jaringan taut silangan antara rantai poliisoprena. Akibatnya kelikatan fasa NR akan meningkat dan menjadi setanding dengan fasa PS dan keadaan ini memungkinkan percampuran antara NR dan PS. Pembentukan gugusan zarah NR juga akan terjadi di sekitar pusat tindak balas radikal dan sebahagian dari radikal akan terkumpul di permukaan zarah yang akan bertindak sebgai pusat aktif. Kewujudan pusat aktif boleh meningkatkan pelekatan antara fasa dalam adunan. Tindak balas ini menyebabkan pembentukan rangkaian taut silang yang luas dan adunan menjadi matang, keras dan kuat. Lagi pesat dan luas pembentukan jaringan taut silangan akan menambahkan lagi kekuatan bahan.

Sifat Mekanik

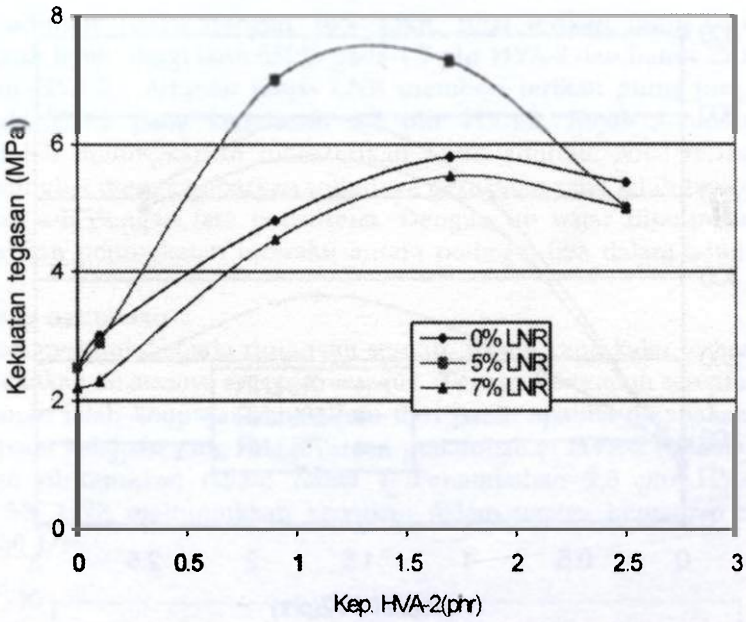
(a) Tegasan

Rajah 2 menunjukkan pengaruh HVA-2 terhadap kekuatan tegasan. Secara amnya nilai kekuatan tegasan meningkat dengan kepekatan HVA-2 dalam adunan ke suatu paras maksimum dan menurun selepas itu. Coran dan Patel (1980) melaporkan yang kekuatan tegangan bagi termoplastik elastomer EPDM/PP meningkat dengan darjah pembentukan taut silang. Apabila sebanyak 5% LNR ditambah ke dalam adunan 60/40, amaun HVA-2 yang diperlukan untuk mencapai kekuatan tegasan maksimum berkurang dari 1.7 phr kepada 1.25 phr. Sebaliknya nilai kekuatan tegasan maksimum meningkat dari 5.8 Mpa kepada 7.4 Mpa. Namun apabila lebih banyak LNR ditambah iaitu 7%, nilai ini turun semula kepada 5.6 Mpa.

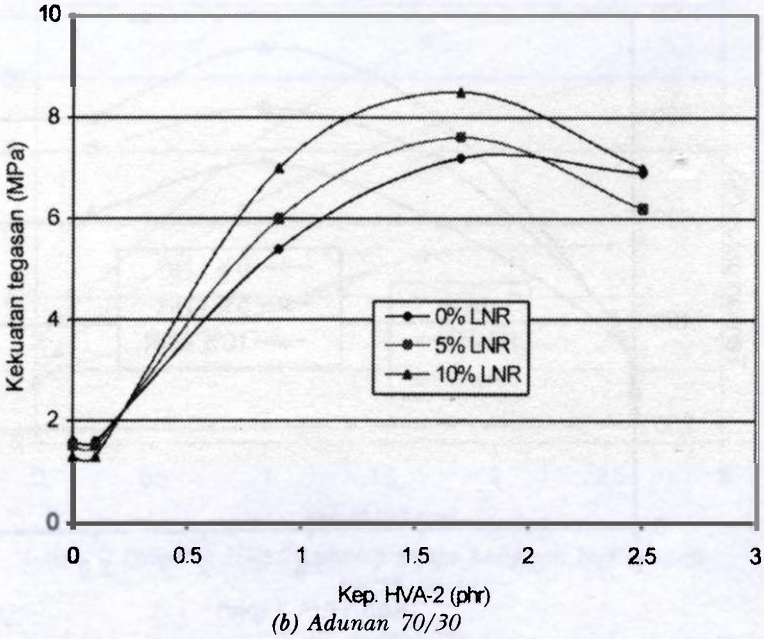
Bagi adunan 70/30, kekuatan tegasan maksimum yang boleh dicapai lebih tinggi iaitu 8.5 Mpa pada kepekatan 1.7 phr HVA-2 dan 10% LNR. Penambahan 5% LNR hanya meningkatkan 5.5% kekuatan tegasan maksimum. Ketiga-tiga adunan yang berkomposisi 70/30 memerlukan 1.7 phr HVA-2 untuk mencapai kekuatan yang maksimum. Menurut Methew dan Tinker (1986), HVA-2 boleh menyebabkan taut silangan dalam fasa getah seterusnya memberi kekuatan tegasan yang lebih tinggi.

(b) Terikan

Pengaruh HVA-2 ke atas terikan putus adalah seperti dipamerkan oleh *Rajah 3*. Graf ini secara amnya menunjukkan kenaikan nilai terikan putus apabila kepekatan HVA-2 dalam adunan bertambah. Nilai terikan putus bagi adunan 60/40 yang dapat dicapai adalah 500% pada kepekatan 1.65 phr HVA-2 dan 5% LNR. Penambahan sebanyak 7% LNR adunan 60/40 menyebabkan terikan putus berkurang kepada 450%. Bagi adunan tanpa LNR, terikan putus yang dicapai meningkat dari 50% kepada 280% apabila ditambah 1.7 phr HVA-2 ke dalam adunan.

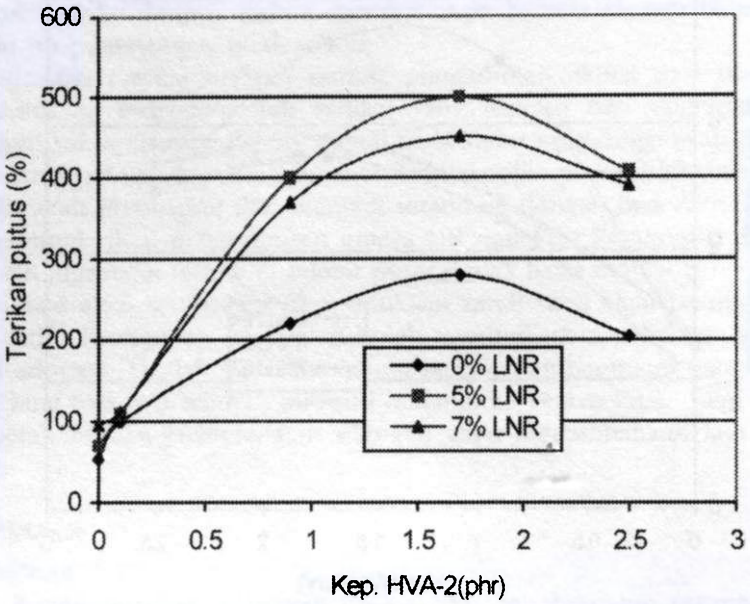


(a) Adunan 60/40

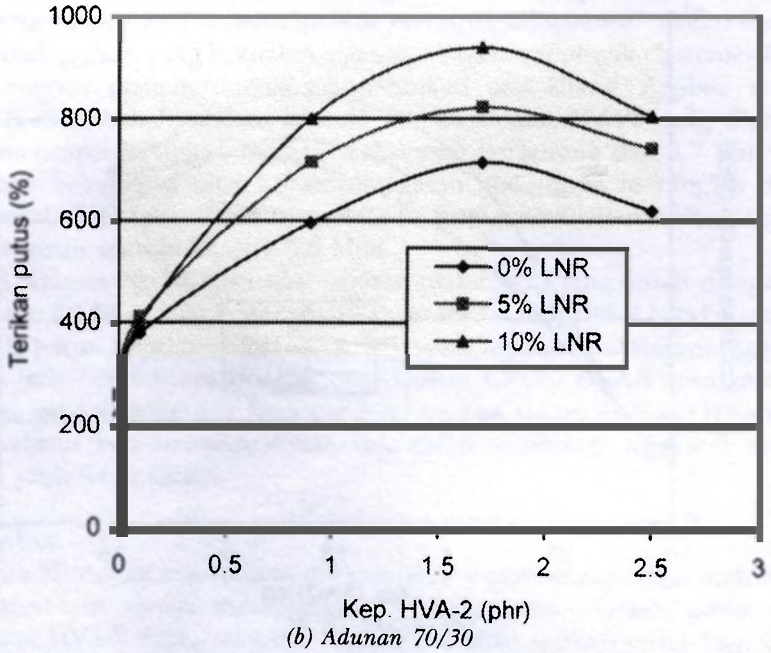


(b) Adunan 70/30

Rajah 2: Pengaruh HVA-2 terhadap kekuatan tegasan adunan NR/PS



(a) Adunan 60/40



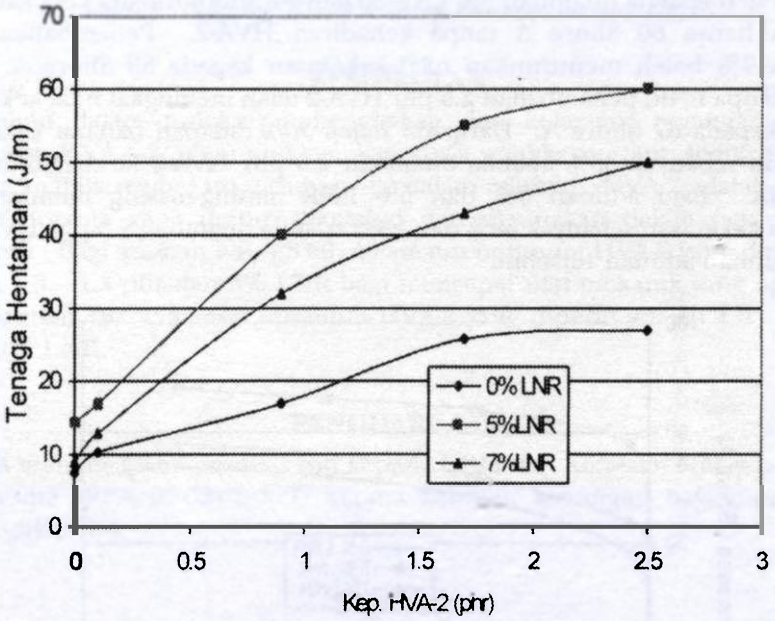
(b) Adunan 70/30

Rajah 3: Pengaruh HVA-2 terhadap terikan putus adunan NR/PS

Bagi adunan 70/30 dengan 10% LNR, nilai terikan putus yang dapat dicapai jauh lebih tinggi iaitu 950% pada 1.7 phr HVA-2 dan hanya 220% tanpa kehadiran HVA-2. Adunan tanpa LNR memberi terikan putus yang rendah iaitu pada 720% pada kepekatan 1.7 phr HVA-2. *Rajah 3* menunjukkan HVA-2 boleh meningkatkan nilai terikan putus adunan. Nilai terikan putus yang meningkat menggambarkan wujudnya perekatan yang lebih baik di antara fasa getah asli dengan fasa polistirena. Dengan itu wajar disarankan HVA-2 menyebabkan peningkatan interaksi antara pelbagai fasa dalam adunan.

(c) Tenaga hentaman

Hentaman merujuk kepada rintangan sesuatu bahan yang kaku terhadap daya yang dikenakan ke atasnya secara mengejut. Tenaga hentaman sesuatu polimer atau adunan ialah keupayaan menahan dari patah apabila dikenakan tegasan (stress) pada kelajuan yang tinggi. Kesan penambahan HVA-2 terhadap tenaga hentaman ditunjukkan dalam *Rajah 4.* Penambahan 2.5 phr HVA-2 pada adunan 5% LNR menunjukkan kenaikan dalam tenaga hentaman dari 14.4 kepada 60 J/m.



Rajah 4: Pengaruh HVA-2 terhadap tenaga hentaman bagi adunan NR/PS 60/40

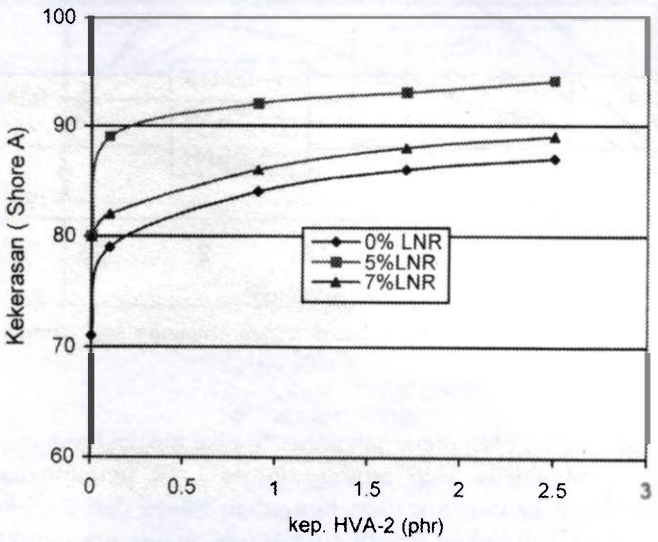
Apabila lebih banyak LNR ditambah iaitu 7% nilai tenaga hentaman menurun kepada 50 J/m. Manakala bagi adunan tanpa LNR penambahan 1.7 phr HVA-2 menyebabkan kenaikan tenaga hentaman hanya dari 7.45 kepada 25.8 J/m. Peranan HVA-2 di dalam pembentukan taut silang dan perekatan antara fasa juga dapat dilihat daripada perubahan nilai tenaga hentaman. Perekatan

antara fasa yang dihasil melalui pencangkukan dan penyebaran domain-domain yang menyeluruh di dalam adunan telah meningkatkan nilai tenaga hentaman.

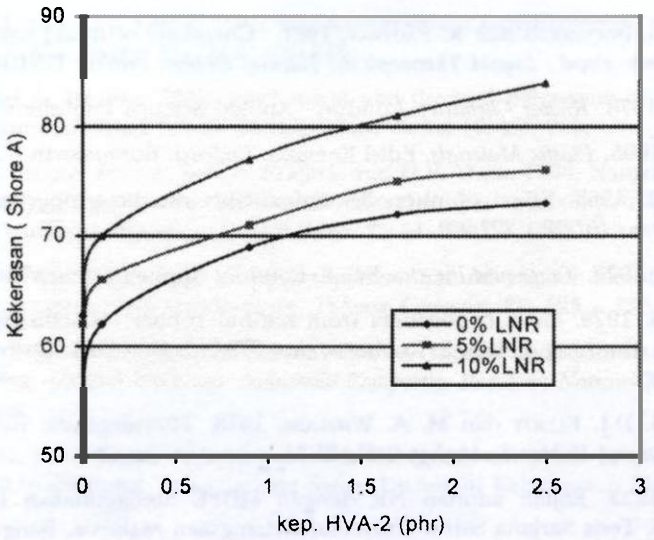
Dalam adunan NR/PP, HVA-2 telah dilaporkan memberi kesan peningkatan kekuatan hentaman adunan (Methew dan Tinker 1986). HVA-2 berkeupayaan untuk menggabungkan PP dan radikal getah semasa proses ricihan untuk memberikan perekatan antara muka yang lebih baik di antara fasa PP dan fasa getah. Pembentukan taut silangan dalam fasa getah juga dipengaruhi oleh HVA-2. Jadi kedua-dua keadaan ini boleh menyebabkan tenaga hentaman adunan NR/PP meningkat. Menurut Bucknal (1977) kekuatan hentaman sesuatu adunan dipengaruhi oleh beberapa faktor seperti perekatan antara fasa, morfologi fasa serta kekuatan kohesif fasa getah.

(d) Kekerasan

Rajah 5 menunjukkan pengaruh HVA-2 terhadap sifat kekerasan bagi adunan NR/PS dan penambahan HVA-2 telah meningkatkan nilai kekerasan Shore A adunan. Bagi adunan 60/40 dan 70/30, nilai kekerasan terus meningkat walaupun pada kepekatan 2.5 phr HVA-2. Nilai kekerasan bagi adunan 60/40 yang dicapai apabila ditambah 5% LNR adalah 94 Shore A pada kepekatan 2.5 phr dan hanya 80 Shore A tanpa kehadiran HVA-2. Penambahan LNR sebanyak 7% boleh menurunkan nilai kekerasan kepada 89 Shore A. Bagi adunan tanpa LNR, penambahan 2.5 phr HVA-2 telah meningkat nilai kekerasan dari 71 kepada 87 Shore A. Daripada *Rajah 5(b)*, didapati bahawa kekerasan meningkat sebanyak 33% apabila ditambah 2.5 phr HVA-2 ke dalam adunan 10% LNR. Bagi adunan 0% dan 5% LNR masing-masing menunjukkan kenaikan kekerasan sebanyak 38% dan 23% apabila ditambah 2.5 phr HVA-2 ke dalam adunan-adunan tersebut.



(a) Adunan 60/40



(b) Adunan 70/30

Rajah 5: Pengaruh HVA-2 terhadap kekerasan adunan NR/PS

KESIMPULAN

Perubahan dalam indeks pembengkakan dan kekuatan regangan dengan komposisi HVA –2 menunjukkan wujudnya rangkaian taut silangan dalam matriks apabila reagen ini dicampur ke dalam adunan. HVA-2 adalah penjana radikal organik yang umum diketahui dan digunakan dalam pemvulkanan getah asli. Bagi adunan NR/PS 60/40 amaun optimum HVA-2 yang diperlukan adalah 1.3 – 1.3 phr dan 5% LNR bagi mencapai sifat mekanik yang optimum. Bagi adunan 70/30 amaun optimum HVA-2 yang diperlu adalah 1.6 – 1.7 phr dan 10% LNR.

PENGHARGAAN

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Low-Cost Optical Fibre Chemical Sensor for Use in Liquid Acid-Base Titration

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ABSTRAK

Instrumen mudah alih untuk penderia kimia gentian optik telah dibina dalam kajian ini menggunakan diod laser sebagai sumber cahaya dan fotodiod keadaan-pepejal sebagai pengesan. Peranti yang berkos rendah ini menunjukkan rangsangan yang baik dan boleh digunakan dalam tiga jenis titratan asid-bes iaitu i.e. asid kuat – bes kuat, asid kuat – bes lemah dan asid lemah – bes kuat. Hasil kajian yang diperoleh menggunakan instrumen ini, didapati memberikan keputusan yang menyamai keputusan yang diperoleh menggunakan pH meter konvensional.

ABSTRACT

A simple low-cost portable optical fibre chemical sensor instrument has been developed in this study by using a laser diode as a light source and solid-state photodiode as a detector. This inexpensive device is shown to have a good response and can be used in three different liquid acid-base titration studies i.e. strong acid-strong base, strong acid-weak base and weak acid-strong base, by using a suitable pH indicator. The results obtained by using this instrument were found comparable with titration data obtained by using conventional pH meter.

INTRODUCTION

Chemical sensing with optical fibres is one of the most interesting of the emerging sensor techniques. Optical fibre chemical sensors permit the determination of a wide range of anions, cations, gases and organic compounds in solution or gas phases (Narayanaswamy 1985). Impetus to develop these sensors has arisen largely from the need to rapidly acquire data such as chemical compositions of process streams in manufacturing plants, *invivo* body fluids monitoring for clinical purposes and gaseous atmospheres, ground and river waters for pollution monitoring. The basic concept and the advantages of chemical sensors based on optical fibres have been discussed by many authors (Narayanaswamy 1985; Seitz 1984; Alder 1986; Narayanaswamy and Sevilla 1988; Wolfbeis 1986; Morris 1989; Narayanaswamy 1993; Saari 1987).

To date, quite a number of optical fibre chemical sensors for pH measurements have been reported in the literature. The pH indicators for these sensors could be either immobilised on a support (Alabbas *et al.* 1989;

Wolfbeis *et al.* 1992; Munkholm *et al.* 1986; Ding *et al.* 1991; Kirkbright *et al.* 1984; Peterson *et al.* 1980; Moreno *et al.* 1990; Gupta and Marma 1998; Xu *et al.* 1998; Grant and Glass 1997; Pilar *et al.* 1997; Wallace *et al.* 1997; McCullosh and Uttamchandani 1997; Bromberg *et al.* 1996; Zhang *et al.* 1996; Netto *et al.* 1995; Motellier *et al.* 1995, Zhang *et al.* 1995, Ding *et al.* 1991) or directly used in a solution form (Benaim *et al.* 1986; Sahrim *et al.* 1996). Zhu *et al.* (1992) has reported a simple optical fibre pH sensor developed by incorporating a pH paper at the tip of bifurcated fiber. The use of immobilised indicators will require the indicators to be immobilised to a kind of solid support such as entrapment in sol-gel film (Ding *et al.* 1991; Gupta and Sharma 1998; Grant and Glass 1997), a membrane[23] or by simple adsorption on a polymeric support (Alabbas *et al.* 1989; Kirkbright *et al.* 1984; Moreno *et al.* 1990). Without immobilisation, the pH indicator was normally directly added to the solution and the colour changes were monitored by the optical fibre probe. The signal obtained from these sensors, i.e. either reflectance (Alabbas *et al.* 1989; Kirkbright *et al.* 1984; Moreno *et al.* 1990; Benaim *et al.* 1986; Sahrim *et al.* 1996; Christian 1994) or fluorescence[10,1117,20,26] are correlated to changes in pH of the solution.

The use of optical fibre chemical sensor to monitor the progress of acid-base titration has earlier been reported by Shahrim *et al.* (1996), Benaim *et al.* (1986) and Moreno *et al.* (1990) The first two papers have reported using free pH indicators without prior immobilisation i.e. phenolftalien and phenol red, respectively. The work of Benaim *et al.* (1986) was on the development of simple optical pH sensor instrumentation with the probe designed for reflectance measurement. However, the type of acid-base titration carried out with this sensor was not mentioned. Shahrim *et al.* (1996) reported the same type of reflectance probe with a fully computerized instrumentation system for acid-base titration between HCl and NaOH. Moreno *et al.* (1990) has been using cresol red immobilised on the anion-exchange resin as an indicator in acid-base titration between NaOH and strong acids (hydrochloric, perchloric and sulphuric acids), and also weak acids (salicylic, hydrofluoric and phosphoric acids). The instrumentation used in this work was a bulky tungsten-halogen lamp fitted in a spectrophotometer with photomultiplier tube as a detector. The measured parameter was the intensity of the light reflected by the resin, which was guided to the detector by the return fibre.

In this paper we presented a simple low-cost portable instrumentation for optical fibre pH sensor. This sensor can be used to monitor the progress of three different types of acid-base titration by using suitable indicators. Different from the reflectance type of sensors which have been previously developed, the design of the sensor used in this study was based on transmission mode.

METHOD

Chemicals and Solutions

Three different pH indicators are used in this study for titration end-point detection i.e. bromotymol blue (strong acid-strong base titration), bromophenol

blue (strong acid–weak base titration) and tymol blue (weak acid-strong base titration). All the pH indicator solutions were prepared by dissolving 0.050 g of the indicator powder in 20% etanol and the solution was 50.00 mL volume in a volumetric flask.

The acid and base used in this study are hydrochloric acid, HCl; acetic acid, CH_3COOH; sodium hydroxide, NaOH and ammonia, NH_3. The acid and base solutions were prepared through appropriate dilution of the supplied solution to produce acid and base solutions of 0.1 M concentration.

The Electronic Circuits

The complete electronic circuit developed in this study is shown in *Fig. 1.* It consists of a signal generator, a light source, a photodiode, a pre-amplifier, a sample-hold circuit, an amplifier and a display unit. The light from a laser diode will be transmitted to the optical fibre and will be guided by the feed fibre to the detection area where the light will be modulated. The modulated light will be guided by the return fibre to the photodiode. Upon detection, photodiode will produce a small electrical current which will be amplified and transferred to voltage by pre-amplifier circuit. The signal will later be passed to the sample-hold circuit which will produce outputs which are proportional to the signals received by the photodiode when the laser diode is on or off. In such a sequence, an interferent from ambient light will be avoided and as a result, the overall output is only a detected laser diode light intensity which has been modulated at detection area. Output from the sample-hold circuit will later go to the amplifier for further signal amplification before being displayed.

Signal generator circuit: The signal generator circuit developed in this study consists of a multivibrator and a driver (*Fig. 2*). The multivibrator device consists of transistor (TR2 and TR3), timing component, resistor (R6 and R7) and capacitor (C3 and C4). Transistor TR1 and TR4 functioned as a driver for laser diode and sample-hold circuit.

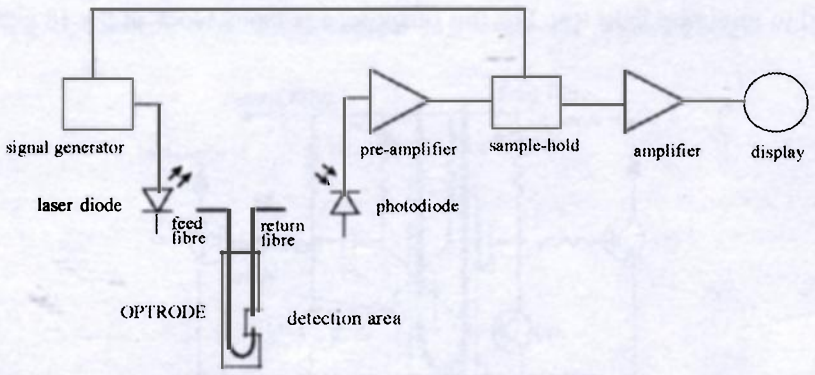


Fig. 1: Electronic circuit for low-cost portable instrument optical fibre chemical sensor

Pre-amplifier circuit: The pre-amplifier circuit used in this study was BIFET operational-amplifier (op-amp) because its input impedance which is high, is suitable for handling small electrical signals. The small electrical current produced by photodiode will flow to the inverting input (-) and then directly to a feedback resistor R_f which will transform the current to output voltage as shown in *Fig. 3 (A)*.

Sample-hold circuit: The components used to build this circuit consist of a diode, capacitor C2 and BIFET op-amp as shown in *Fig. 3 (B)*. An electrical signal in the form of voltage pulse will be filtered and will charge the capacitor C2. When the laser diode is off, the transistor collector TR1 and diode anode will be at zero level and therefore, no signal will pass the diode. Therefore, only a signal pulse is allowed to pass this circuit. The charge at the capacitor C2 will be discharged only through resistor R2 because the input impedance at the op-amp non-inverting input (+) is very high. The discharge duration is governed by the values of resistor R1 and capacitor C2 used. In this study, the discharge time is 1 second.

Amplifier circuit: The output signal produced by the sample-hold circuit is not big enough to be displayed and an amplifier circuit is needed for further amplification of the signal. The amplifier circuit consists of bipolar op-amp as shown in *Fig. 3 (C)*. The values of R_f and R_i used in this circuit will determine the amplification values, E_i. The end of R_i was connected to V_R which, having potential difference of (+) and (-), ensures that the Vo values could be adjusted.

Construction of Optical Electrode (Optrode)

The optical fibre used for optrode construction is of multimode silica type with core diameter of 1 mm and numerical aperture, NA of 0.47. Before using the optical fibre, its surface was polished by using lapping sheets to produce a smooth and flat surface.

The design of the optrode used in this study is shown in *Fig. 4*. A mould made from plasticine was used to incorporate the optical fibre in the resin epoxy. The end of both the feed and return fibres in the detection has to be aligned to minimise light lost. For this purpose, a perspex block of 5 x 18 x 20 mm

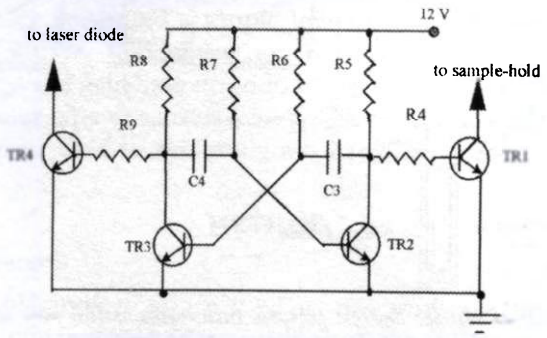


Fig. 2: Electronic circuit for signal generator

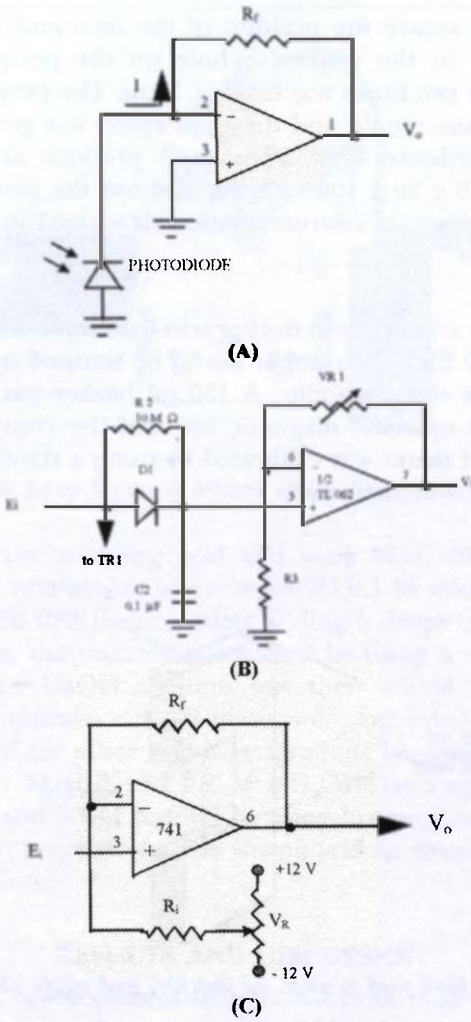


Fig. 3: Electronic circuit for pre-amplifier (A), sample-hold (B) and amplifier (C)

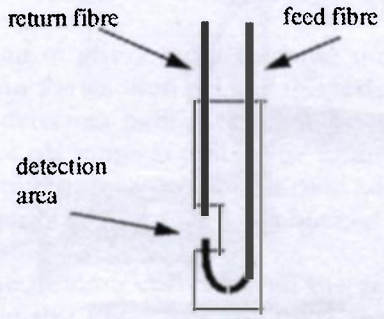


Fig. 4: The design of the optical fibre optrode

(*Fig. 5*) was used to secure the position of the feed and return fibres. Both fibres were inserted in the respective hole on the perspex block and the distance between the two fibres was fixed at 1mm. The perspex block was later placed in the plasticine mould and the resin epoxy was gradually poured into the mould. The hardened resin epoxy will produce an optrode with an approximate size of 5 x 20 x 100 mm. *Fig. 6* shows the picture of the low-cost portable optical fibre sensor instrumentation developed in this study.

Procedures

The instrumentation arrangement during acid-base titration process is as shown in *Fig. 7*. The optical fibre instrument has to be warmed up for about 15 min before use for better signal stability. A 150 ml beaker was used as a titration container where the optrode, magnetic bar and the conventional pH meter were placed. The pH meter was calibrated by using a standard buffer solution of pH 4.0 and pH 9.0 before use.

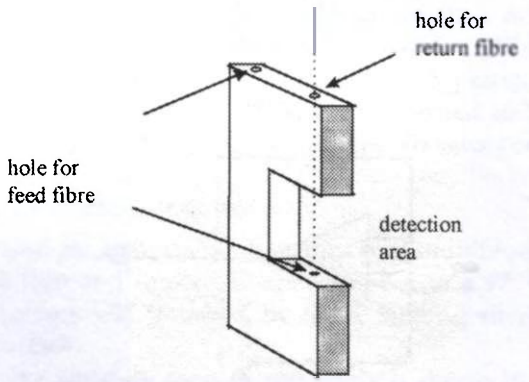


Fig. 5: A perspex block used to align the feed fibre and return fibre in the optrode

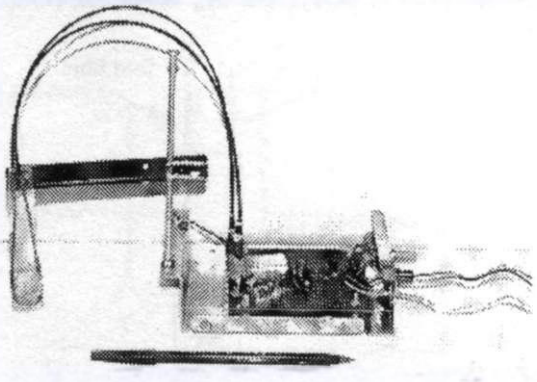


Fig. 6: Instrumentation for portable optical fibre chemical sensor

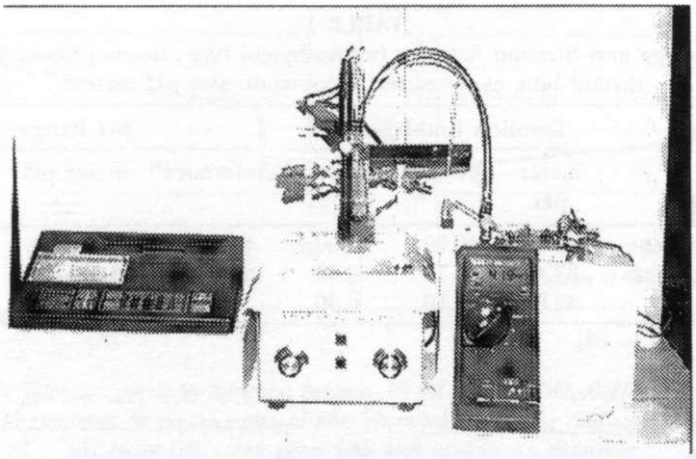


Fig. 7: Experimental set-up for use of portable optical fibre sensor in acid-base titration

For titration between strong acid and weak base, 40 mL of 0.1 M HCl solution was placed in a beaker whereas NaOH 0.1 M solution which was used as a titran was placed in a burette. After adding 5 drops of bromothymol blue in the acid solution, the mixture was stirred by using a magnetic stirrer at a constant speed. The NaOH solution was then added gradually and both readings from the optrode and pH meter were recorded. The same titration process was repeated for other acid-base titrations i.e. titration between strong acid–weak base (0.1 M HCl and 0.1 M NH_4OH) and strong base-weak acid (0.1 M CH_3COOH and 0.1 M NaOH) by using bromophenol blue and thymol blue as an indicator, respectively. The strong acid or strong base was used as a titran in each titration.

RESULTS AND DISCUSSION

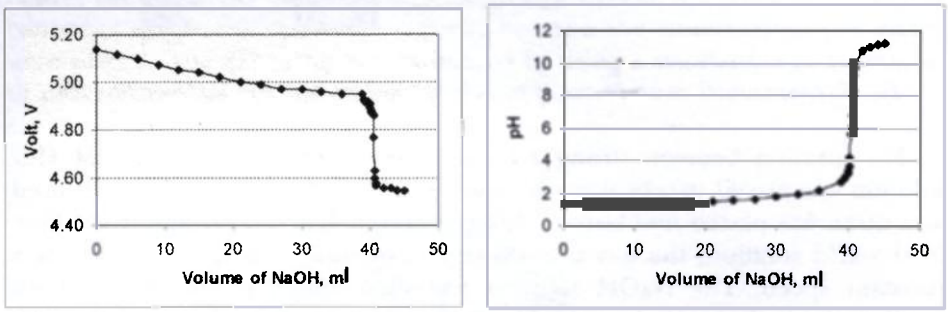
Bromothymol blue, bromophenol blue and thymol blue were chosen in this study because their colour changes (yellow to blue) are suitable for light source used in this study i.e red laser diode. The significant contrast in the colour changes of the indicators used is also important to ensure it could be easily detected by the voltmeter.

The sensor was found to give a rapid response time (in a range of a few seconds) upon changes in the solution pH and this is due to the optical method of measurements with detectors having a sub-microsecond response. Table 1 summarizes the values of pH range as well as the titration end-point for all the indicators used in this study as measured by optrode and pH meters. As shown, the pH range and the titration end-point data obtained from both optrode and pH meters are comparable.

Figs. 8 – 10 show the titration curves for all the acid-base titrations which have been carried out in this study. The voltmeter reading from the optrode was found to change with changes in the solution pH. This change is basically

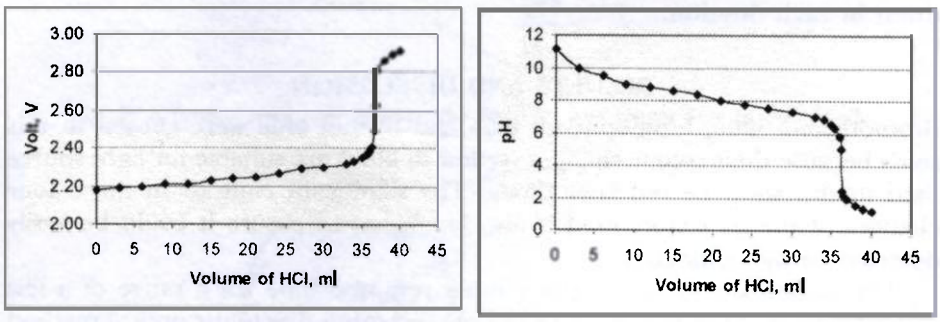
TABLE 1
The pH range and titration data for bromothymol blue, bromophenol blue and thymol blue as measured by optrode and pH meters

pH indicator	Titration End-Point, mL			pH Range		
	meter pH	optrode	different	reference[31]	meter pH	optrode
bromothymol blue	40.70	40.70	0.00	6.0 – 7.6	4.2 – 9.2	4.0 – 8.0
bromophenol blue	37.40	37.70	0.30	2.8 – 5.0	3.0 – 6.2	2.7 – 6.3
thymol blue	42.80	43.10	0.30	7.0 – 9.6	6.8 – 9.4	7.2 – 9.2



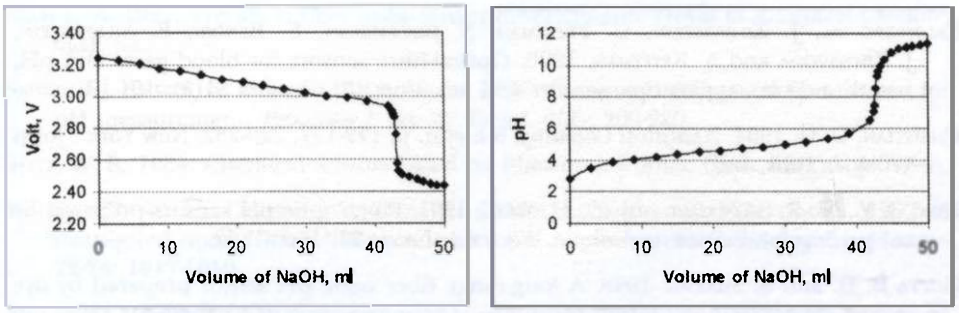
(A) (B)

Fig. 8: Titration curve for titration between 40 mL HCl, 0.1M and NaOH, 0.1M measured by portable optical fibre



(A) (B)

Fig. 9: Titration curve for titration between 40 mL NH_3, 0.1M and HCl, 0.1M measured by portable optical fibre chemical sensor (A) and conventional pH meter(B), when bromophenol blue was used as an indicator



(A) (B)

Fig. 10: Titration curve for titration between 40 mL CH_3COOH, 0.1M and NaOH, 0.1M measured by portable optical fibre chemical sensor (A) and conventional pH meter (B), when tymol blue was used as an indicator

due to the changes in the colour of the solution when the titran was added gradually to the solution. When the end-point of the acid-base titration is reached, the colour change is completed and therefore an abrupt change occurs in the volt reading as shown in *Figs. 8 – 10 (A)*. A similar shape of titration curves was also observed for acid-base titration between HCl and NaOH monitored by optical fibre pH sensor based on reflectance measurement (Moreno *et al.* 1990). These changes correspond to similar changes observed in the pH reading as shown in *Figs. 8 – 10 (B)* when the pH of the solution was monitored by using conventional pH glass electrode.

CONCLUSION

The low-cost portable optical fibre sensor has been successfully developed in this study. The optrode could be used to monitor the progress of liquid acid-base titration between strong acid-strong base, strong acid-weak base and weak acid-strong base. The opto-electronic components used were a laser diode and photodiode detector with no optical filtering elements.

ACKNOWLEDGEMENT

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Studies on Heavy Metal Accumulations in Green-Lipped Mussel *Perna viridis* from Malaysia by Using Multiple Linear Stepwise Regression Analysis

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ABSTRAK

Kepekatan Cd, Cu, Pb and Zn dalam tisu lembut kupang *Perna viridis* daripada 15 lokasi di Malaysia didapati rendah berbanding data di rantau ini. Namun demikian, sampel-sampel Kuala Perlis dan Kg. Pasir Puteh patut diberi lebih perhatian kerana lokasi-lokasi tersebut dikenal pasti menerima logam berat hasil daripada aktiviti manusia. Analisis regresi 'multiple linear stepwise' didapati berguna untuk mencari parameter yang paling berpengaruh dalam menentukan biopenimbunan logam berat dalam kupang *P. viridis*. Keputusan analisis tersebut menunjukkan bahawa parameter yang terpilih adalah lebih kurang konstan tidak kira pelbagai model yang digunakan dalam prosedur. Oleh yang demikian, analisis regresi 'multiple linear stepwise' boleh dicadangkan sebagai cara statistik alternatif dalam menentukan parameter yang paling berpengaruh yang menyebabkan biopenimbunan logam berat di dalam *P. viridis* dan invertebrata yang lain.

ABSTRACT

The concentrations of Cd, Cu, Pb and Zn in soft tissue of green-lipped mussel *Perna viridis* from 15 sampling sites in Malaysia were found to be not serious but low when compared to regional data. However, samples from Kuala Perlis and Kg. Pasir Puteh should be given more attention since these locations were identified to have received anthropogenic metals. The multiple linear stepwise regression analysis was found to be useful in finding the most influential statistical parameters affecting heavy metal accumulation in *P. viridis*. The regression analyses indicated that the parameters were rather constant regardless of different sets of models being included in the procedure. Therefore, the multiple linear stepwise regression analysis can be proposed as an alternative statistical method for determining the most important variables affecting heavy metal accumulations in *P. viridis* as well as in other invertebrates.

Keywords: *Perna viridis*, heavy metals, multiple linear stepwise regression analysis

INTRODUCTION

Statistical analysis in biological data has always been an important step in interpreting the data. There are a number of different ways as well as different statistical means in performing the analysis. Regression analysis is a statistical

method to determine if there are relationships between the variables studied and it is also useful in the study of the shape of the curve of the relationship and allows researchers to look for the reasons for the relationships (Snedecor and Cochran 1980).

Multiple Linear Stepwise Regression Analysis (MLSRA) was applied here to find an empirical equation relating to the dependent variable(s) (metal concentration) that has a high value of r^2 (coefficient of determination) using only the most important independent variables (Bethea *et al.* 1985). Stepwise regression requires the assumption that there is only one best equation (Bethea *et al.* 1985) and that the procedure in the Statistical Analysis System (SAS) program will find it. The general forward selection procedure in SAS begins by using the independent variables one at a time, comparing the corresponding reduction in the error sum of squares with some pre-set criterion (a = 0.15 is the default for PROC STEPWISE) and then either retaining or rejecting the term (Bethea *et al.* 1985). STEPWISE procedure is a modified forward selection method in which the F-value for each term in the model is calculated, compared to the corresponding tabulated values of F and rejected if it is not significant for the model (Bethea *et al.* 1985). Then the next term is added to the model and the process is repeated. In the present study, MLSRA was carried out by using independent variables which included non-ratios and ratios. The non-ratio independent variables included shell length (LE), shell width (WI), shell height (HE), flesh dry weight (FW), shell dry weight (SW) and sex while the ratio independent variables included condition index (CI), width : height (WH), width : length (WL), length : height (LH) and shell thickness (ST). Ratios express relationships between quantitative variables and thereby aid in summarizing and interpreting statistical data (Whitmore *et al.* 1973).

In addition, a comparison between a procedure which included all independent variables and a procedure which included only independent ratios was discussed. These two models were compared based on all populations collected from 15 stations. They were termed as inter-station MLSRA. The third model which only included one population from one sampling site (Pasir Panjang) was termed as intra-station MLSRA which included 27 female and 29 male individuals. Inter-station and intra-station models were calculated in order to find out if similar independent variables were selected as the most influential parameters affecting metal accumulation in both flesh and shell of green-lipped mussel *P. viridis*. The comparison of these different models was made to examine if MLSRA can be recommended as a good statistical means in finding out the most important factors affecting the accumulations of Cd, Cu, Pb and Zn of *P. viridis*.

The dependent variables for the MLSRA included Cd, Cu, Pb and Zn concentrations in both flesh and shell of the mussel *P. viridis*.

MATERIALS AND METHODS

There were 15 sampling sites from Malaysian coastal waters (*Fig. 1*) in this study. All the samples were collected between May and September 1998. Information about reproductive cycles of *P. viridis* in the sampling sites revealed that in the Straits of Johore the natural spats could be found throughout the year, mainly from November to February and May to June (Shamsudin 1992; Aldon and Buendia 1998). Koh *et al.* (1991) reported that the settlement of pelagic *P. viridis* in the coastal waters of the Straits of Johore peaked during the inter-monsoon months of November and April.

The mussels at each sampling site were analysed according to sex, and shell lengths for each site and sex are given in Table 1. In the present study, between 20 and 32 individuals (male and female) were used to represent one station (Table 1) and this sample size was within what was usually reported in many studies using bivalves as biomonitors (Phillips and Rainbow 1993, Hung *et al.* 2001, Yap *et al.* 2002).

In general, the sampling sites could be classified depending on their surroundings. For instance, a site at Kuala Perlis could be categorized as a jetty, sites at Langkawi are fish culture areas whilst Kg. Pasir Puteh is a marina and near urban and industrial areas. Other areas are believed to have agricultural activities.

All individuals were measured for their LE, WI and HE with vernier calipers. Further, dried shell weight and dried flesh weight were determined for calculating

Fig. 1: Map showing sampling stations of green-lipped mussel Perna viridis

TABLE 1
Mean cm ± standard error (SE) of shell length, shell width, shell weight, fresh dry weight and shell dry weight per individual of mussel *P. viridis* from 15 sampling stations

	Sampling locations	Sex	N	Shell length
1	K. Perlis	Female	10	3.64 ± 0.17
	Perlis.	Male	12	3.40 ± 0.12
2	Tanjung Rhu,	Female	10	7.00 ± 0.06
	Langkawi.	Male	15	7.08 ± 0.07
3	Sangkar Ikan,	Female	9	7.12 ± 0.23
	Langkawi.	Male	11	6.87 ± 0.25
4	Tanjung Dawai,	Female	7	7.96 ± 0.52
	Kedah.	Male	13	8.25 ± 0.20
5	Penang Island,	Female	8	6.60 ± 0.52
	Penang.	Male	15	7.59 ± 0.19
6	Bagan Lalang,	Female	10	9.38 ± 0.18
	Selangor.	Male	15	8.93 ± 0.20
7	Lukut,	Female	14	9.53 ± 0.18
	N. Sembilan.	Male	11	9.23 ± 0.19
8	Pasir Panjang,	Female	12	8.40 ± 0.24
	N. Sembilan.	Male	13	9.18 ± 0.49
9	Pantai Kundor,	Female	16	8.89 ± 0.09
	Malacca.	Male	14	8.52 ± 0.11
10	Anjung Batu,	Female	13	9.31 ± 0.12
	Malacca.	Male	16	9.17 ± 0.14
11	Pantai Lido,	Female	18	6.06 ± 0.15
	Johore.	Male	14	5.80 ± 0.13
12	Kg. Pasir Puteh,	Female	15	6.89 ± 0.44
	Johore.	Male	16	5.92 ± 0.40
13	Kg. Tg. Batu,	Female	10	2.51 ± 0.04
	Pahang.	Male	12	2.57 ± 0.04
14	Trayong,	Female	8	10.97 ± 0.50
	Sabah.	Male	11	10.98 ± 0.42
15	Kuala Penyu,	Female	7	7.20 ± 0.15
	Sabah.	Male	15	6.66 ± 0.10

Note: Besides what is shown in Table 1, samples from Pasir Panjang were also analysed from 2.0 to 11.0 cm of shell lengths.

the allometric ratios with LE, WI and HE. The soft tissues and shells of *P. viridis* were analyzed according to sex. The whole soft body was carefully removed by deshelling the mussel with a stainless knife. The dry weight of the individual soft body was determined by drying the whole body tissues for 72 h at 105°C to a constant weight (Mo and Neilson 1994). At each location, every individual's inner nacreous shell layer was ground into a fine powder in an agate mortar and pestle, before being homogenised and weighed for digestion with an accuracy of 0.1 mg. The samples were digested in concentrated nitric acid and

assayed for heavy metals by using a flame atomic absorption spectrophotometer (AAS) Perkin-Elmer Model 4100. The data are presented in mg/g dry weight basis. Mutiple-level calibration standards were analysed to generate calibration curves against which sample concentrations were calculated. Standard solutions were prepared from 1000 mg/l stock solution of each metal (MERCK Titrisol).

A quality control sample was routinely run through during the period of metal analysis. To avoid possible contamination, all glassware and equipment used were acid-washed and the accuracy of the analysis was checked with the standard addition testing method. Procedural blanks were prepared and the metal concentrations in the blanks were negligible. Percentages of recoveries were 95% for Cu, 92% for Zn, 110% for Cd and 92.5% for Pb.

The statistical analyses were done by using the Statistical Analysis System Version 6.0 (SAS Institute 1987) software package. Data transformation was carried out to stabilize the variance and the lack of normality to produce a frequency distribution that was nearer to normal distribution (Sheldon and Haick 1981). After all data were converted into the desired values, the data would be transformed to $\log_{10}$ (X) according to the procedure of Boulton and Lake (1990).

The models are as follows :-

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + \_\_ + b_NX_N$$

where Y = dependent variable
X_1 -X_N = independent variable
b_0 = intercept
b_1 - b_N = slope for each independent variable

The allometric ratios calculated were WH, WL, LH, ST and CI. ST, considered to be an age measure in bivalves (Cossa *et al.* 1980; Frew *et al.* 1989), was calculated with the following formula :-

$$ST\ (g/cm^2) = \frac{\text{shell weight (g)}}{\text{shell length (cm)} \times \text{shell height (cm)}}$$

CI, regarded as an indication of the mussel's individual physiological state reflecting the amount of stored energy which may be considered as the animal's metabolic response to environmental conditions (Peddicord 1977), was calculated as shown below:-

$$CI\ (g/cm^3) = \frac{\text{total fresh dry weight (g)}}{\text{shell height (cm)} \times \text{shell length (cm)} \times \text{shell width (cm)}} \times 1000$$

RESULTS AND DISCUSSION

Heavy Metal Concentrations in Mussels

Since the international 'Mussel Watch' approach uses total soft tissue of marine mussels to monitor the heavy metal contamination in the coastal waters (Goldberg 1975; Phillips and Segar 1986; Phillips and Rainbow 1993), only heavy metals in the soft tissue of *P. viridis* were discussed in the present study. From the 15 locations, the ranges of metals varied from 0.19 to 1.56 µg/g dry weight for Cd, 5.78 to 15.14 µg/g dry weight for Cu, 1.17 to 8.27 µg/g dry weight for Pb and 46.78 to 145.95 µg/g dry weight for Zn *(Fig. 1)*. These ranges of Cd, Cu, Pb and Zn were all comparable and lower than those reported in *P. viridis* from Thailand (Sukasem and Tabucanon 1993), India (Senthilnathan *et al.* 1998) and Hong Kong (Wong *et al.* 2000).

Samples from Kuala Perlis were found to have high concentrations of Cd (1.56 mg/g dry weight) and Pb (8.27 mg/g dry weight). Kuala Perlis has a lot of boating activities and some port operations (Ismail *et al.* 1991). These have become the potential sources of heavy metal contamination in the area. To support our results, some crabs were reported to have elevated levels of heavy metal in Kuala Perlis by Ismail *et al.* (1991). Samples from Kg. Pasir Puteh were found to have the highest Cu concentration (15.14 µg/g dry weight). Since industries, shipping and urban sewage were considered the major sources of metal contamination, the elevated Cu level found in Kg. Pasir Puteh was an interesting result. Samples from Trayong of Sabah had high concentrations of Cd (1.18 µg/g dry weight) and Zn (121.79 µg/g dry weight). Only fish and mussel aquacultural activities were found in this area and the potential sources of this metal contamination are still unknown. Other sampling sites showed relatively low concentrations of heavy metal since most of these areas practised agricultural activities. However, these levels were not considered serious.

Finding the Most Influential Factors by Using Mutiple Linear Stepwise Regression Analysis

Inter-station Comparison

All populations from 15 stations (inter-station) were tested to determine the most important known factors affecting metal accumulation (Tables 2, 3). The regression equations observed appear to fall into four distinct categories. First, Cu, Pb and Zn concentrations in the flesh and Pb concentration in the shell were all significantly influenced by sex besides other variables (Table 2). The above-mentioned metals were higher in females, as indicated by the negative sign in sex (Lobel *et al.* 1989). Second, it was noted that CI was the most influential variable in both flesh (Cu and Zn) and shell (Cd, Pb and Zn). However, Zn-flesh was negatively influenced by CI.

Third, Cu and Pb in both flesh and shell were inconsistently influenced by ratios such as WH, WL and LH. It was somewhat surprising that ST was not found to influence the Cu and Pb concentrations. Probably, ST could be replaced by physiological terms such as growth rate (WL) and CI. Since CI is

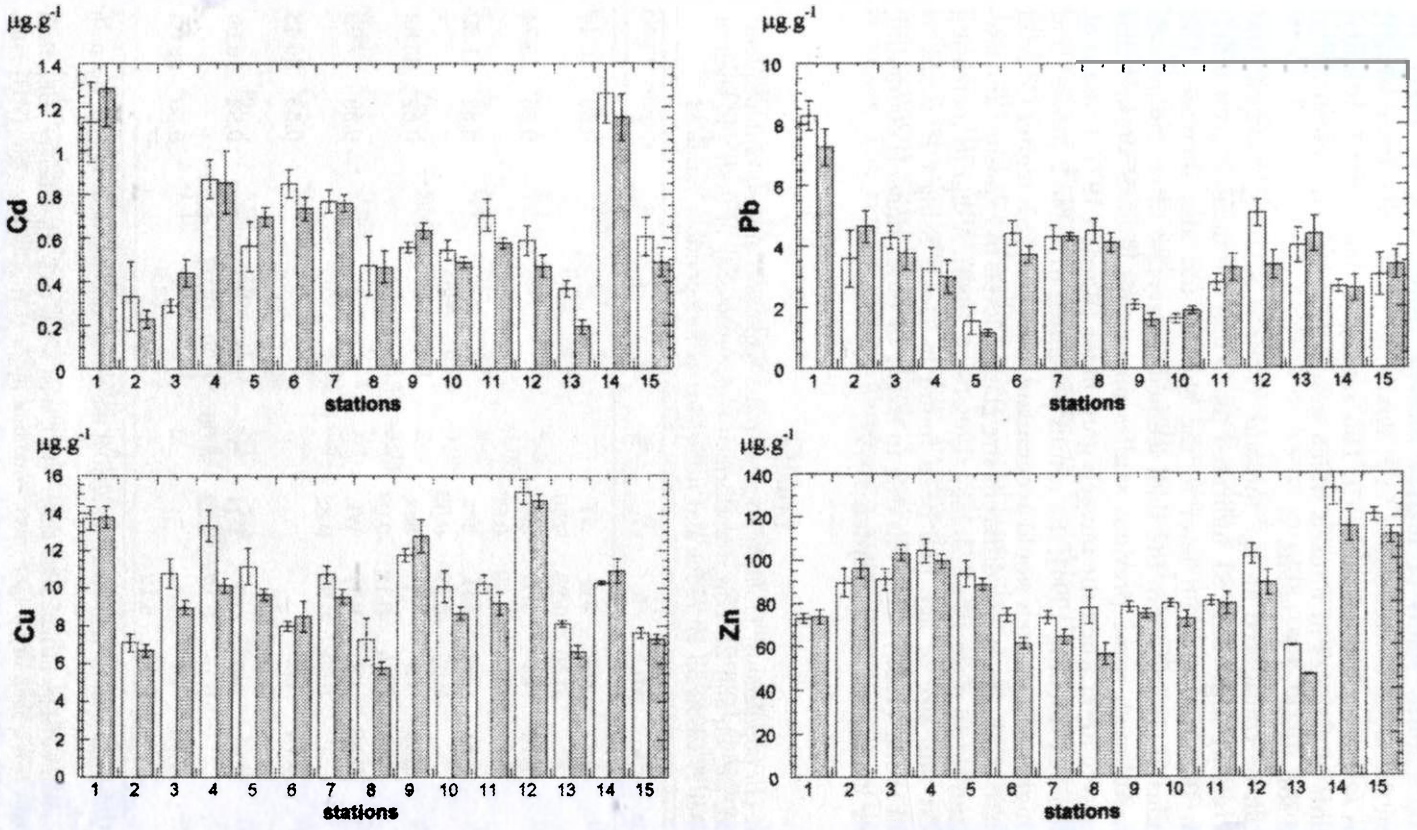

Fig.2: Concentrations of Cd, Cu, Pb and Zn in total soft body (mean mg/g dry weight ± standard error) of mussel P. viridis *sampled from 15 sampling stations (▭ : female; ■ : male).*

Note: Anjung Batu (St 10),Bagan Lalang (St 6), Kg. Pasir Puteh (St 12), Kg. Tg. Batu (St 13), Kuala Penyu(St 15), Kuala Perlis (St 1), Lukut (St 7), Pantai Kundor (St 9), Pantai Lido (St 11), Pasir Panjang (St 8), Penang (St 5), Sangkar Ikan (St 3), Tg. Dawai (St 4), Tg. Rhu (St 2), Trayong (St 14).

indicative of maturity status of marine mussels (Broman *et al.* 1991), its influence on the metal concentration in *P. viridis* was reasonable. Interestingly, Zn-flesh was negatively influenced by CI. This suggested that high CI indicated great amounts of gametes in mussels which would yield less Zn concentration in the total soft tissue of *P. viridis*.

As ST can be considered an age measure (Frew *et al.* 1989), Cd-flesh and Pb-shell increasing with rising ST indicated that when the mussels grew older, Cu level in the flesh and Pb level in the shell would also increase. The concentrations of Cu and Pb in the shell seemed to increase when the mussels grew older. Although some previous studies on marine bivalves showed that metal levels were lower when the mussels grew older (Boyden 1977; Cossa *et al.* 1980; Swaileh and Adelung 1994), the results presented in Table 2 showed that the older (higher ST) mussels would accumulate more Cd. This higher Cd level in mussel flesh might be due to different size classes, levels of exposure and site-dependent individual mussel's physiology (Lobel *et al.* 1989). A slightly increasing trend for Cd with size or age had been found in Hong Kong's *P. viridis* by Cheung and Wong (1992). This was due to larger or older mussels containing considerable amounts of Cd (Boyden 1977).

TABLE 2
Inter-station (15 populations) multiple linear stepwise regression analysis of dependent variables (Cd, Cu, Pb and Zn Log_{10} transformed concentrations) in flesh and shell of mussel *P. viridis* on all ratios and non-ratios as independent variables

Heavy Metals		a	b_1	b_2	b_3	b_4	b_5	b_6	b_7	r	F
Flesh	Cd	-0.27	HE	FW	ST					0.39	16.44
			-0.50	0.28	0.50						
	Cu	1.19	CI	WL	Sex					0.36	15.74
			0.16	-1.09	-0.06						
	Pb	0.31	WL	LH	Sex					0.32	11.89
			2.09	-0.19	-0.06						
	Zn	2.59	CI	ST	Sex					0.62	66.83
			-0.45	-0.18	-0.03						
Shell	Cd	-0.63	CI	WH	WL					0.39	17.99
			0.29	0.28	0.82						
	Cu	0.74	LE							0.33	39.43
			0.10								
	Pb	2.91	WI	CI	WH	WL	LH	ST	Sex	0.52	16.59
			0.15	0.17	2.29	-5.00	-0.79	0.08	-0.02		
	Zn	-1.10	CI	WL						0.39	28.06
			0.37	3.12							

Note: All variables significantly ($P < 0.001$) influenced the metal concentrations in both the flesh and shell.
Independent variables include shell length (LE), shell width (WI), shell height (HE), flesh dry weight (FW), shell dry weight (SW), condition index (CI), width : height (WH) ratio, width : length (WL) ratio, length : height (LH) : ratio, shell thickness (ST) and sex.
r is the coefficient of correlation.

WL ratios seemed to influence most of the metals in this study, namely Cu and Zn in the flesh and Cd, Cu and Zn in the shell. This ratio is important as it relates to the growth rate and relative age of the mussel (Kautsky 1982). Seed (1968) suggested that mussels in a given environment reach a maximum shell length beyond which no further linear growth could occur. Owing to shell erosion, the shell would continue to thicken and expand resulting in a lowering of the CI. The expansion of the shell which is necessary to prevent the ST from being squashed, is in the form of increased shell width. Therefore, an increase in WL ratio indicated a mussel with increased growth rate and an increased relative physiological age.

Comparison Between Two Models: All Variables (Non-ratios and Ratios) and All Ratios

Two different sets of independent variables (Tables 2, 3) were investigated to evaluate the differences and how well the MLSRA models performed. Independent variables in Table 2 included all ratios and non-ratios as independent variables while Table 3 only included all ratios as independent variables. The results showed almost the same independent variables as the

TABLE 3

Inter-station (15 populations) multiple linear stepwise regression analysis of dependent variables (Cd, Cu, Pb and Zn Log_{10} transformed concentrations) in flesh and shell of mussel *P. viridis* on all ratios as independent variables

Heavy Metals		a	b_1	b_2	b_3	b_4	b_5	b_6	b_7	r	F
Flesh	Cd	-0.97	CI	ST						0.35	22.16
			0.22	0.85							
	Cu	1.19	CI	WL	Sex					0.36	15.74
			0.16	-1.09	-0.06						
	Pb	0.31	WL	LH	Sex					0.32	11.89
			2.09	-0.19	-0.06						
	Zn	2.59	CI	ST	Sex					0.62	66.83
			-0.45	-0.18	-0.03						
Shell	Cd	-0.63	CI	WH	WL					0.39	17.99
			0.29	0.28	0.82						
	Cu	0.88	WL	ST						0.35	20.57
			-0.32	0.09							
	Pb	1.32	CI	LH	ST	Sex				0.45	18.86
			0.14	-0.05	0.22	-0.02					
	Zn	-1.10	CI	WL						0.39	28.06
			0.37	3.12							

Note: All variables significantly ($P < 0.001$) influenced the metal concentrations in both the flesh and shell.
Independent variables include condition index (CI), width : height (WH) ratio, width : (WL) length ratio, length : height (LH) ratio, shell thickness (ST) and sex.
r is the coefficient of correlation.

most important factors affecting Cu, Pb and Zn levels in the flesh and Cd and Zn levels in the shell, for both regression models each with its respective r value. For example, Zn-flesh was significantly affected by CI (negative), ST (negative) and sex (negative) with r= 0.62 in both regression models. Therefore, these almost similar results suggested that multiple linear stepwise regression analysis was performing as expected and that the independent factors selected were reliable. It is logical that some equations did not include the same influential factors. For instance, Cd in flesh and Cu and Pb in shell were found to have different significant independent variables. Flesh-Cd was significantly influenced by shell height, flesh dry weight and ST as shown in Table 2, whereas as shown in Table 2, flesh-Cd was significantly influenced by CI and ST.

In the two models, it was noted that the r values obtained were low which indicated that there may be other independent variables that had not been included in this study of what we think are the most important factors to influence metal accumulation in the mussel *P. viridis*.

Intra-station and Inter-station Comparisons

Mussels with a wide range of age and size (shell length: 2.0-11.0cm) in a single population at Pasir Panjang (station 5) were tested to find out the most important factors known to influence their heavy metal concentrations in the flesh and shell (Table 4). The results suggested that the factors which significantly influenced each metal accumulation in intra-station analysis were not similar to those found in inter-station analysis. This may be due to the different size classes of mussels in this intra-station population receiving the same amount of environmental inputs while inter-station mussels were receiving different environmental inputs and the maximum potential shell length at each station was not similar (Lobel *et al.* 1991). However, flesh-Cu and shell-Pb seemed to be influenced by the same known important factors for both intra-station and inter-station populations namely CI and sex. This may be indicative that CI and sex were the two important factors affecting the accumulation of flesh-Cu in the mussel *P. viridis* regardless of different statistical models provided that CI and sex were included in the stepwise procedures. Similarly, ST and sex were two important variables affecting the accumulation of Pb-shell in the three models (Tables 2, 3 and 4). These results agreed with an intra-station study by Lobel *et al.* (1991) who found that sex, FW, CI and WH ratios were four of the most important independent variables which influenced the concentration of metals in the flesh of the blue mussel *M. edulis*. In this study, ST and sex were also two important factors which influenced Pb accumulation in the shell of *P. viridis*.

CONCLUSION

The ranges of heavy metal concentrations in all sampling sites were found to be comparable and lower than the regional data in the soft tissue *P. viridis*. Kuala Perlis and Kg. Pasir Puteh were found to be potentially receiving anthropogenic metals. In the identification of the most influential known

TABLE 4
Intra-station (Population Pasir Panjang) multiple linear stepwise regression analysis of dependent variables (Cd, Cu, Pb and Zn Log_{10} transformed concentrations) in flesh and shell of mussel *P. viridis* on all ratios and non-ratios as independent variables

Heavy Metals		a	b_1	b_2	b_3	b_4	b_5	b_6	b_7	r	F
Flesh	Cd	-0.06	FW							0.88	88.67
			-0.77								
	Cu	1.62	CI	Sex						0.45	3.04
			0.29	-0.08							
	Pb	0.36	HE	ST						0.70	11.85
			-4.39	2.28							
	Zn	1.96	FW	Sex						0.84	30.06
			-0.20	-0.07							
Shell	Cd	1.45	WI	ST	Sex					0.68	6.86
			-1.82	1.58	-0.11						
	Cu	0.71	LH							0.77	38.94
			0.44								
	Pb	1.64	FW	ST	Sex					0.63	5.26
			-0.23	0.71	0.07						
	Zn	1.84	HE	FW	ST					0.80	14.32
			-1.51	-0.21	1.43						

Note: All variables significantly ($P< 0.001$) influenced the metal concentrations in both the flesh and shell.
Independent variables include shell length (LE), shell width (WI), shell height (HE), flesh dry weight (FW), shell dry weight (SW), condition index (CI), width : height (WH) ratio, width : length (WL) ratio, length : height (LH) : ratio, shell thickness (ST) and sex.
r is the coefficient of correlation.

factors in the accumulation of metals, there may be other important factors, besides the known factors included in the present study, affecting metal accumulation in the tissues and shells of the mussels. The factors affecting heavy metal accumulation in mussels seemed to be variable whether they were ascertained as intra-station or inter-station. However, the same known variables ascertained in the three models indicated that MLSRA may be proposed as an alternative statistical approach to determine the most influential factors out of a number of factors which were known to affect metal accumulation in the green-lipped mussel *P. viridis* in particular and in other invertebrates in general.

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Investigation on the Chemical Constituents of the Leaves of *Ficus elastica* Roxb. and Their Antimicrobial Activity

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ABSTRAK

Empat sebatian (emodin, sukrosa, morin dan rutin) diasingkan daripada daun-daun *Ficus elastica* Roxb. Struktur-struktur sebatian tersebut dibentuk menggunakan teknik-teknik spektroskopik dan perbandingan dengan data yang diterbitkan. Sebatian-sebatian tersebut ditutup untuk aktiviti antimikrobia ke atas dua spesies bakteria, *Bacillus cereus* (Gram-positif) dan *Pseudomonas aeruginosa* (Gram-negatif) dan empat spesies fungus dengan menggunakan kaedah resapan cakera. Sebatian-sebatian itu menunjukkan aktiviti antibakteria tetapi tiada aktiviti antifungus diperhatikan ke atas organisma-organisma yang diuji.

ABSTRACT

Four known compounds (emodin, sucrose, morin and rutin) were isolated from the leaves of *Ficus elastica* Roxb. The structures of the compounds were established by spectroscopic techniques and by comparison with published data. The compounds were screened for antimicrobial activity against two species of bacteria, *Bacillus cereus* (Gram-positve) and *Pseudomonas aeruginosa* (Gram-negative) and four species of fungi by using the disc diffusion method. The compounds showed antibacterial activity but no antifungal activity was observed against the tested organisms.

Keywords: *Ficus elastica*, emodin, morin, antimicrobial, cytotoxicity

INTRODUCTION

Many *Ficus* species are commonly used in traditional medicine to cure various diseases. They have long been used in folk medicine as astrigents carminatives, stomachics, vermonicides, hypotensives, antihelmintics and anti-dysentery drugs (Trivedi *et al.* 1969). Previous phytochemical studies on the genus showed the presence of flavonoids (Mohammad 1991), alkaloids (Ikhlas *et al.* 1993), organic acids (Ilyas 1990) and triterpenes (Beat 1990).

Ficus elastica (Moraceae) is a widely-spread evergreen tree up to 30 m tall. The leaves are 7-20 cm long, with smooth edges and blunt pointed tips. The leaves are about a foot long and are thick with deep green colour. The plant is known locally as "india-rubber tree" (Burkill 1966). No chemical and biological investigations have been reported on *Ficus elastica.* In this study we report the

chemical constituents and antimicrobial activity of the compounds isolated from the plant.

MATERIAL AND METHODS

Plant Material

The leaves of *Ficus elastica* were collected from the Universiti Putra Malaysia campus in April 2000 and identified by taxonomist Umi Kalsom Yusuf from UPM. A voucher specimen (16291) was deposited at the Herbarium of the Department of Biology, UPM.

General Material and Methods

Melting points were determined on a Kofler hot-stage microscope melting point apparatus and were uncorrected. IR and UV spectra were obtained on Perkin-Elmer lambda model 1330 and 20 spectrometers respectively. ^{1}H and ^{13}C-NMR were recorded on JEOL JNM-GSX 400 spectrometer. Mass spectra were obtained using GCMS-QP 5050 A Shimadzu mass spectrometer. Column chromatography and analytical TLC were carried out using silica gel, Merck 230 - 400 mesh and Merck 70-230 mesh.

Extraction and Isolation of Compounds

The leaves of *Ficus elastica* were air-dried, ground and subjected to extraction with petroleum ether, chloroform and methanol successively. The solvents were then removed under reduced pressure to give the following extracts as shown in Table 1.

TABLE 1
The extracts of the leaves of *Ficus elastica*

Material (g)	Solvent	Colour and nature of extracts	Yield of extracts (g)
Leaves (2500)	petroleum ether	yellowish-brown, semi-solid	9.7
	chloroform	dark- green, solid	15.1
	methanol	dark-brown, solid	48.4

Isolation of Emodin (I)

The crude chloroform extract (12.0 g) was subjected to silica gel column chromatography using gradient solvent mixtures of 100% petroleum ether, petroleum ether/chloroform (1:1), 100% chloroform and chloroform/methanol (5:2) to give 15 fractions. Fractions 9 and 12 which gave the same R_F values (0.35, chloroform:methanol, 3:1) on TLC were combined together and resubjected to column chromatography with chloroform and methanol, (1:1) to give emodin (I) as reddish orange crystals (42.3 mg) with melting point 258-260°C (Gow-chin *et al.* 1998, 259-260°C).

Isolation of Sucrose (II)

The methanol extract (48.4 g) was redissolved in 95% aqueous methanol (500 ml) and then extracted successively with n-hexane, chloroform, ethyl acetate and n-butanol. The n-butanol extract was chromatographed on silica gel column chromatography with chloroform:methanol gradients of increasing polarities followed by rechromotography of fractions 5-8 to yield 11 fractions. Fraction 5 afforded yellow solid material which was washed with methanol to give yellowish crystals and reextracted with absolute ethanol to give sucrose (37.8 mg) with melting point 193-194°C (Pouchert and Camphell 1974, 190-192°C) and R_f value 0.37 (100% ethyl acetate).

Isolation of Morin (III)

Fractions 24-29 from the above column were combined and rechromatographed to yield a yellow powder. Recrystallisation from acetone gave morin as pale yellow needles (55.6 mg) with melting point 303-304°C (David and Milne 1995, 303.5°C).

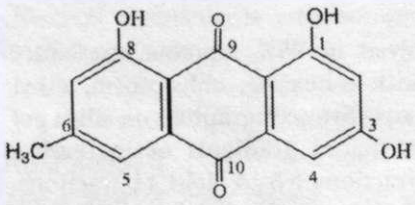
Isolation of Rutin (IV)

Fractions 33-36 obtained from the above column were also combined and resubjected to column chromatography eluted with 100% chloroform with increasing amounts of methanol to give 14 fractions. Fractions 8-10 gave a yellow solid material which was recrystallised in acetone to yield rutin (IV) as yellow crystals (38.5 mg) with melting point 212-214 (C (Hill *et al.* 1991, 214-215 (C).

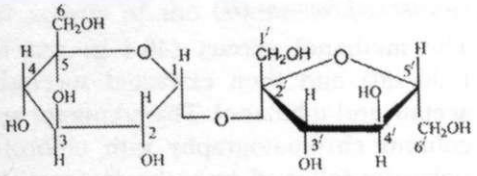
Antimicrobial Assay

The microorganisms were obtained from the culture collection of the Department of Biotechnology, Universiti Putra Malaysia. The stock cultures were grown on potato dextrose agar (PDA) for 24 h at 28°C at which time the cells were harvested by centrifugation (4°C, 2000 rpm, 3 min.). The cells were washed and suspended in sterile 0.9% saline to give a final concentration of 10^5-10^6 CFU/mL using a haemocytometer (Bergeys 1957). The bacterial strains used were *Bacillus cereus* NRRLUI-1447 and *Pseudomonas aeruginosa* UI-60690 while the antifungal strains were *Aspergillus ochraceus* NRRL 398, *Candida lipolytica* ATCC 2075, *Sacchromyces cereviseae* NRRL 2034 and *Sacchromyces lipolytica.*

Antimicrobial activity of the isolated compounds were tested using the disc diffusion method according to Bauer *et al.* (1966). The discs were prepared by impregnating them in an ethanolic solution of each sample (10 mg/mL). They were then evenly spaced out on the agar surface previously inoculated with the suspension of each microorganism to be tested. Standard discs of nystatin (50 g/discs) and streptomycin sulphate (25 g/discs) were used as standard antifungal and antibacterial agents respectively. The plates were incubated at 37°C for 24 h and the antimicrobial activity was recorded by measuring the diameter of the clear inhibition zones around each disc.



(I)



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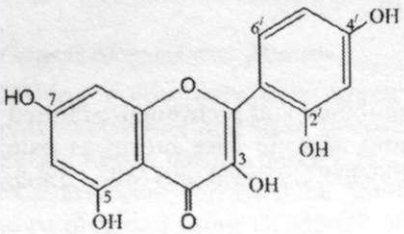

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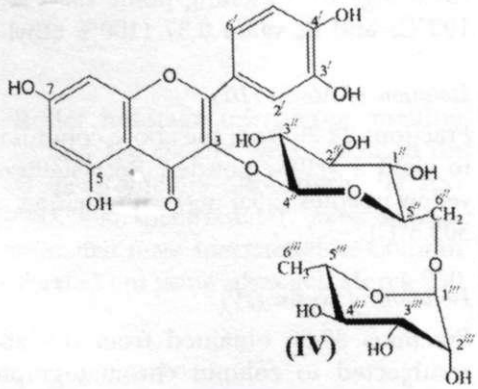

(IV)

RESULTS AND DISCUSSION

Four compounds have been successfully isolated from *Ficus elastica* by column chromatography. These compounds have been identified to be emodin (I), sucrose (II), morin (III) and rutin (IV).

There were four protons in the aromatic region of the ^{1}H-NMR spectrum appearing as singlets at δ 7.56, 7.30, 7.05 and 6.65 and assigned for proton at C-4, C-5, C-2 and C-7 respectively. The two sharp singlets in the downfield region at δ 12.25 and 12.18 were assigned to the two chelated hydroxyl groups at positions 1 and 8. A small broad singlet at δ 10.62 indicated the presence of the free hydroxyl group at C-3, whereas the singlet at δ 2.44, integrated for three protons, was assigned to a methyl substituent.

The ^{13}C-NMR spectrum indicated the presence of fifteen carbons in the structure. The two carbonyl carbons occurred at very low fields, 189.8 and 181.5 ppm. The high field signal at 21.5 ppm is due to the methyl group at C-6. The mass spectrum gave a molecular ion peak at m/z 270 which corresponded to the molecular formula $C_{15}H_{10}O_5$. On comparison, the spectral with published data (Suraj 1989; Parkas and Anderson 1982) suggested the structure to be 1, 3, 8-trihydroxy-6-methyl-9, 10-anthracenedione (emodin).

Compound II was identified as sucrose based on IR, ^{1}H, ^{13}C-NMR, MS and on comparison with published data.

The aromatic region of the ^{1}H-NMR spectrum of compound III showed the presence of five aromatic protons. A pair of doublets (J=1.7 H_z) occurred at δ 8.06 and 6.92. These were assigned to the *meta* protons at H-6 and H-8, respectively. Another doublet observed at δ 7.26 with coupling constant 2.0 Hz was assigned for proton at H-3′. The proton at H-6′ occurred as doublet due to coupling with proton at H-5′ which in term coupled to H-3′. The three singlets occurring at very low field regions at δ 12.62, 10.79 and 9.85 (×2) could be assigned to the four hydroxyl groups at C-5, C-7 and C-2′ and C-4′ (overlapped) respectively. The ^{13}C-NMR spectrum assignments were based on a DEPT experiment. The DEPT spectrum also showed the presence of fifteen carbon atoms consisting of ten quartenary carbons (C-7, C-9, C-4′, C-2, C-2′, C-5, C-4, C-3, C-1′ and C-10) and five methine carbons (C-6, C-8, C-3′, C-5′ and C-6′). The mass spectrum showed the presence of a molecular ion at m/z 302 corresponding to the molecular formula $C_{15}H_{10}O_7$ in agreement with a flavonol skeleton (Markham 1982) and structure of morin (i.e., 3, 5, 7, 2′, 4′ -pentahydroxyflavone).

The UV spectrum of compound IV showed absorptions at λ_{max}360.0 and 265.0 nm, indicating the presence of 3-O-substituted flavonol skeleton (Geissan, 1962). The ^{1}H-NMR spectrum clearly establishes the presence of chelated hydroxyl groups with the occurrence of a sharp singlet at δ 12.61 for 5-OH. In addition, the ^{1}H-NMR spectrum also showed the presence of five protons which appeared as doublet at δ 7.56 (J = 2.2 H_z), 6.81 (J = 2.2 H_z), 6.39 (J = 2.2 H_z) and 6.18 (J = 2.2 H_z) and were assigned to H-2′ and H-6′ (overlapped), H-5′, H-8 and H-6 respectively. H-1″ for glucosyl was observed as doublet at δ 5.33. The one singlet peak at δ 4.36 revealed the presence of one proton at H-1‴ for rhamnosyl, in which the methyl group of rhamnosyl appeared at δ 1.02.

The carbon assignments were made by DEPT experiment. The spectrum showed the presence of twenty-seven carbons consisting of ten quartenary carbons (C-4, C-7, C-9, C-5, C-2, C-10, C-4′, C-3′, C-3 and C-1′), fifteen methine carbons (C-6′, C-5′, C-2′, C-6, C-8, C-1‴, C-1″, C-3″, C-5″, C-2″, C-4‴, C-4″, C-3‴, C-2‴ and C-5‴), one methyl carbon at C-6‴ and one methylene carbon at C-6″. The mass spectrum showed the presence of a molecular ion at m/z 610 corresponding to the molecular formula $C_{27}H_{30}O_{16}$. The structure of this compound was thus established as 5, 7, 3′, 4′-tetrahydroxy 3-O-rahmanoglucosyl (rutin).

The four compounds isolated showed antibacterial activity towards the *Bacillus cereus* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative). The activity ranged between weak, moderate and strong based on the diameter of inhibition zones. However, no antifungal activity was observed against the four species of fungi (Table 2).

TABLE 2
Antimicrobial activity of compounds isolated (concentration 100 µg/ml, methanol) from *Ficus elastica*

compound	bacteria		fungi			
	B. cereus	*P. aeruginosa*	*A. ochraceous*	*C. lipolytica*	*S. areviseae*	*S. lipolytica*
emodin	+ +	+ +	-	-	-	-
sucrose	+	+	-	-	-	-
morin	+ + +	+ + +	-	-	-	-
rutin	+ + +	+ + +	-	-	-	-

B. = Bacillus P. = Pseudomonas A. = Aspergillus C. = Candida
S. = Sacchromyces - no inhibition (0 mm) + weak inhibition (1-9 mm)
+ + medium inhibition (10-14 mm) + + + strong inhibition (15-19 mm)

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Resistance of CCA and Boron-Treated Rubberwood Composites Against Termites, *Coptotermes curvignathus* Holmgren

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ABSTRAK

Kajian telah dijalankan untuk menilai ketahanan papan komposit kayu getah yang dirawat dengan kuprum-kromium-arsenik (CCA) dan boron terhadap serangan anai-anai, *Coptotermes curvignathus* Holmgren. Bahan yang digunakan ialah papan tatal berorientasi (OSB) berperekat fenol-formaldehid (PF), papan serpai (particleboard) berperekat melamin-urea-formaldehid (MUF) dan papan venir laminasi (LVL) berperekat urea-formaldehid (UF). Larutan (CCA, 0.5-4.0% w/w, berat kering ketuhar serpai) dan sebatian boron setara asid borik (BAE, 0.5-1.5% w/w) disemburkan ke atas adunan serpai berperekat semasa pembuatan papan komposit. LVL dan sebahagian daripada OSB dan papan serpai tidak dirawat dan digunakan sebagai kawalan bagi tujuan perbandingan. Blok-blok ujian berukuran 25 mm x 25 mm x 12 mm dipotong dari setiap papan komposit dan didedahkan kepada 1.0 ± 0.05 g (10% jenis askar dan 90% pekerja) di dalam botol ujian selama empat minggu (ASTM D:3345-92). Peratusan kehilangan berat blok-blok ujian dan kadar kematian dikira. OSB yang dirawat dengan CCA pada kepekatan 2.0% mempunyai nilai kehilangan berat yang terendah (3.0%) dibandingkan dengan OSB yang dirawat pada lain-lain kepekatan (nilai kehilangan berat 4.1%-6.8%) dan papan tanpa rawatan (18.4%). Nilai kehilangan berat untuk OSB yang dirawat menggunakan larutan 1.0% dan 1.5% BAE ialah masing-masing 6.9% dan 3.8%. Tahap ketahanan papan serpai terhadap serangan anai-anai meningkat 77% apabila ia dirawat dengan 1% BAE. Purata nilai kehilangan beratnya adalah 6.1%. Di antara papan-papan komposit kayu getah tanpa rawatan, OSB berperekat PF mempunyai daya ketahanan tertinggi terhadap serangan anai-anai. LVL berperekat UF lebih tahan daripada papan serpai berperekat MUF. Nilai kehilangan berat untuk kedua-dua papan ini adalah masing-masing 19.3% dan 26.3%. Di akhir tempoh ujian, kematian anai-anai yang diletakkan bersama blok-blok yang dirawat dengan bahan pengawet ialah 100%, manakala kematian anai-anai yang diletakkan bersama papan komposit dan kayu getah pejal yang tidak dirawat ialah di antara 0-16%.

ABSTRACT

A study was conducted to evaluate the resistance of copper-chromium-arsenic (CCA)–and boron-treated rubberwood composites against subterranean termite, *Coptotermes curvignathus* Holmgren. The materials tested were phenol formaldehyde (PF)-bonded oriented strand board (OSB), melamine urea formaldehyde (MUF)-bonded particleboard and urea formaldehyde (UF)-bonded laminated veneer lumber (LVL). Solutions of CCA (0.5-4.0%, w/w of oven dry weight of particles) and boron compounds (boric acid equivalent

(BAE), 0.5 – 1.5% w/w) were separately sprayed onto the furnish during boards manufacturing. The untreated LVL, some of the OSB and particleboard were served as control for comparing purposes. Test blocks, 25 mm x 25 mm x 12 mm were cut from each of the composites and were exposed to 1± 0.05 g termites (10% soldiers and 90% workers) in test bottles for 28 days (ASTM D: 3345-92). Weight losses of the blocks and termite mortality were calculated. OSB treated with 2.0% CCA had the lowest weight loss value (3.0%) compared to those treated with other concentrations (weight loss values = 4.1-6.8%) and those untreated (18.4%). The weight loss for 1.0% and 1.5% BAE-treated OSB blocks were 6.9% and 3.8%, respectively. The resistance of particleboards against termite attack increased by 77% when they were treated with 1% BAE. The mean weight loss value for these boards was 6.13%. Among the untreated rubberwood composites, PF-bonded OSB had the highest resistance against termite attack. UF-bonded LVL was significantly more resistant to termite attack than MUF-bonded particleboards. The weight loss value for the former was 19.3% and the latter was 26.3%. At the end of the test periods, the mortality of termites that were placed together with the treated blocks was 100%, whereas for those placed together with the untreated composites and solid rubberwood, the mortality ranged from 0-16%.

Keywords: Particleboard, oriented strand board, laminated veneer lumber, copper-chromium-arsenic, boric acid, *Coptotermes curvignathus*

INTRODUCTION

Currently in Malaysia, rubberwood has been extensively used for the production of various boards such as particleboard, medium density fibreboard, and cementboard. These products are generally less susceptible to biodeterioration agents compared to solid wood (Behr 1972), unless used in situations where exposure to moisture or risk of deterioration is likely. Biodeterioration may then occur.

Incorporation of boron compounds or copper-chromium-arsenic (CCA) in urea formaldehyde and melamine formaldehyde-bonded particleboard, and phenol formaldehyde-bonded oriented strand board (OSB) had enhanced the resistance of the boards against white rot fungus (Zaidon *et al.* 1998a; 1998b; 2001; Wong 2000). However, the resistance of these composites against termite attack has not been investigated. This study is crucial since there was a rather alarming report of high incidences of subterranean termites attacking wooden cabinets (Sajap 1992).

This paper discusses the resistance of boron- and CCA-treated rubberwood PF-bonded OSB, boron-treated MUF-bonded particleboard and laminated veneer lumber against subterranean termites.

MATERIALS AND METHODS

Sample Preparation

Oriented Strand Board (OSB), particleboard and Laminated Veneer Lumber (LVL) which were manufactured from rubberwood were used in this study. The OSB was bonded with phenol formaldehyde (PF) with 5% resin. The board density was about 700 kg/m^3. The OSB was treated separately with copper-chromium-arsenic (CCA) and with boron compounds (a mixture of boric acid and disodium tetraborate decahydrate (borax); 1 : 1.54 parts. The concentration levels for CCA treatment were 0.5%, 1.0%, 2.0% and 4.0% (w/w of oven-dried weight of particles) whilst for boron compounds, they were 1.0% and 1.5% boric acid equivalent (BAE) (w/w of oven-dried weight of particles). Each solution was sprayed onto the furnish during blending.

The particleboard was bonded with 11% (w/w) melamine urea formaldehyde (MUF). The board density was 650 kg/m^3. Boron solutions at 0.5% and 1.0% (boric acid equivalent, BAE) were incorporated into the furnish during blending of the particles. LVL bonded with 11% (w/w) urea formaldehyde (UF) was also used in this study.

Durability of Composite Panels Against Termites

The test on durability of the treated composite panels and LVL against subterranean termites, *Coptotermes curvignathus* Holmgren was carried out in the laboratory using the method specified in ASTM D3345-93 (Anon 1993). The efficacy of the treatment was evaluated based on the percent weight loss of the materials due to termite attack. Five test blocks (each from different treatment levels, and composite types) 25.4 mm by 25.4 mm by 12.0 mm were randomly selected and conditioned in the laboratory until they reached constant weights. The weights were measured and the blocks were placed in test bottles filled with sand. The bottles, together with their contents, were sterilised at 120°C for 2 h.

About 1 ± 0.05 g termites (*Coptotermes curvignatus*) comprising 10% soldiers and 90% workers were introduced in each of the test bottles. They were then conditioned in a conditioning room maintained at 25 ± 2°C and 65-75% relative humidity overnight. The bottles were then covered with a black paper and kept at room temperature (25-27°C) for a period of four weeks. At the end of the test period, the blocks were removed and cleaned. They were again left in the conditioning room until their weights were constant. The percentage weight loss $(W_a - W_b) / W_a) \times 100$ from the conditioned weight before (W_a) and after exposure (W_b) was calculated. The percentage mortality of termites, $(N_o / N_i) \times 100$, in the test bottle was also calculated based on the number of dead (N_o) and the original number (N_i) of termites. The activities of termites during the testing period were also observed and recorded.

Data were analysed statistically to determine differences in weight losses and mortality rates amongst the composite products and between the preservatives.

RESULTS AND DISCUSSION

Durability of Composite Products

The mean weight loss values for preservative-treated and untreated rubberwood composite blocks after 4 weeks of exposure to subterranean termites are shown in Table 1. The percent increase in resistance of treated boards and the untreated ones and the mean mortality of termites are also listed.

PF-bonded Oriented Strand Board – All the OSB blocks tested were attacked by termites as reflected by the weight loss values. Untreated blocks showed significantly higher weight loss values (18.4%) when compared to those treated blocks (3.8-6.9%). For CCA-treated blocks, the weight loss values reduced as the concentration level of CCA used in the treatment increased from 0.5% to 2.0%. The 0.5% CCA-treated OSB blocks had a mean weight loss value of 6.8%, followed by 1.0% CCA-treated blocks (5.0%) and 2.0% CCA-treated blocks (3.0%). Higher weight loss (4.1%) was found on blocks treated with 4% CCA compared to those treated with 2.0% CCA. However, the difference was not significant. The average weight loss value for untreated OSB was 18.4%. The same trend was also observed for boron-treated OSB. Boards which had been treated with 1.0% and 1.5% boric acid equivalent (BAE) solution had mean weight loss values of 6.91% and 3.82%, respectively.

TABLE 1
Mean weight loss of sample blocks of preservative-treated and untreated rubberwood composites and percent mortality after 28 days exposure to subterranean termites (*Coptotermes curvignathus*)

Sample blocks	Preservative Treatment (%, w/w)	Mean[1] Weight loss (%)	Increase in resistance against untreated boards (%)	Mortality (%)
PF-bonded	-	18.40[a2] ± 2.17	-	16.2
OSB	CCA-0.5%	6.80[b] ± 2.47	63	100
	CCA-1.0%	5.03[c] ± 1.89	72.3	100
	CCA-2.0%	3.04[d] ± 0.92	83.5	100
	CCA-4.0%	4.14[d] ± 2.92	77.5	100
	BAE-1.0%	6.91[b] ± 1.61	62.4	100
	BAE-1.5%	3.82[d] ± 1.14	79.2	100
MUF-bonded	-	26.30[a] ± 1.74	-	0
Particleboard	BAE-0.5%	11.59[b] ± 2.57	55.9	100
	BAE-1.0%	6.13[c]± 0.96	76.7	100
UF-bonded LVL	-	19.26 ± 3.00	-	0
Solid rubberwood	-	80.3 ± 4.12	-	0

[1] Means of 5 sample blocks

[2]Means followed by the same letter are not significantly different (a = 0.05) using Duncan Multiple range test.

The results indicated that the incorporation of both boron compounds and CCA in the rubberwood composites increased their resistance against termite attack. As can be seen in Table 1, CCA treatment increased the resistance of the OSB against termite attack by 63-83.5%, whilst BAE treatment increased the resistance by 62.4-79.2%. The resistance against termite attack apparently increased with an increase in concentration of CCA or boric acid used in the treatments.

MUF-bonded Particleboard – The presence of boron in the particleboard also reduces the destruction caused by the termites. As indicated in Table 1, the mean weight loss values (6.13%-11.59%) for the treated particleboard were significantly lower than the values (26.3%) for the untreated boards. The resistance against termite attack increased with the increase in BAE concentration during treatment, i.e., about 60% and 77% for those treated with 0.5% and 1.0% BAE, respectively.

Between CCA and boron compounds, the former was more effective to protect rubberwood composites against termites. At 1% (w/w), the CCA-treated OSB lost only about 5% of its weight, whilst boron-treated OSB and particleboard lost 6-7%. Although the preservatives are effective in controlling the termite attack, an undesirable green colour found on the surface of the CCA-treated boards reduces the aesthetic value. Boron compounds, on the other hand, adversely affect some of the strength and adhesion properties of the composite panels (Zaidon *et al.* 1998a, 1998b; Wong 2000). Treatment at higher concentrations of boron compound rendered more brittle and rigid flakes (Lloyd and Manning 1995), which induced dimensional instability to the board (Wong 2000).

Untreated Rubberwood Composites – The difference in weight loss values among the untreated composites is also presented in Table 1. All untreated composites showed lower mean weight loss than solid wood. Solid rubberwood had a mean weight loss value of 87%. Amongst the untreated composites, MUF-bonded particleboard (weight loss = 26.3%) was the least resistant whilst PF-bonded OSB (18.4%) was the most resistant towards subterranean termites. This shows that the resins used to bond the boards had an influence on the resistance of the boards. Kajita and Imamura (1991) stated that the resistance of the bonded composites towards termites was due to the inability of termites to digest the materials.

Termite Mortality

PF-bonded OSB – The termite activities and mortality throughout the test period was observed daily. The cumulative mortality of termites in the test bottles containing treated and untreated OSB blocks is illustrated in *Fig. 1.* During the first four days of exposure, about 2 to 10 dead termites were seen in the bottles containing preservative-treated OSB. The mortality of termites increased drastically after the fourth day. 100% mortality was noted in the bottles containing 1% and 2% CCA-treated OSB blocks on the fifth and sixth days. A longer period was taken, i.e., 15 and 18 days, to reach 100% mortality

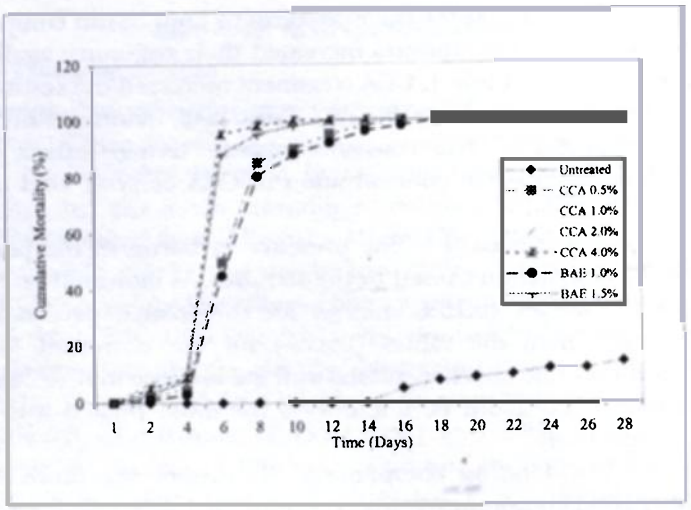

Fig. 1: Cumulative mortality of termites when placed together with treated and untreated OSB blocks in test bottles for up to 28 days

in the bottles containing 0.5% CCA-treated and 1-1.5% BAE-treated blocks. For the untreated OSB blocks, 100% survival was recorded up to 14 days of exposure, but at the end of the test period (28 days), 16.2% of the termites were dead.

MUF-bonded particleboard – *Fig. 2* shows the cumulative mortality of termites in the test bottles containing treated and untreated particleboard blocks. For CCA-treated particleboards, the mortality of termites was only seen after 2 days of exposure. The mortality increased markedly after the fourth and eighth days for particleboard treated with 1.0% and 0.5% BAE, respectively. 100% mortality was recorded after 12 days of exposure for particleboard treated with low concentration of BAE and after 20 days for those treated at a higher concentration. All the termites in the bottles containing untreated particleboard and LVL survived until the end of the test period.

Grace *et al.* (1992) reported that wood blocks treated with more than 0.35% BAE killed all termites within 3 weeks and resulted in 10% weight loss of the blocks. The low mortality of termites for the treated blocks at the early stage of exposure was probably due to the availability of suppliants in the termites which enabled them to survive. At the later stage, the mortality was attributed to the reaction of the toxicant which was ingested by the termites (Mauldin and Karl 1996). The results indicate that the survival periods for the termites were very much related to the toxicity and the chemical loadings in the composite products (Gay and Wetherly 1958; Mauldin and Karl 1996).

Untreated Rubberwood Composites – At the end of the test period, among the untreated composites, dead termites were found only in the bottles containing PF-bonded OSB (*Fig. 1*). The mortality rate increased gradually, and after 28

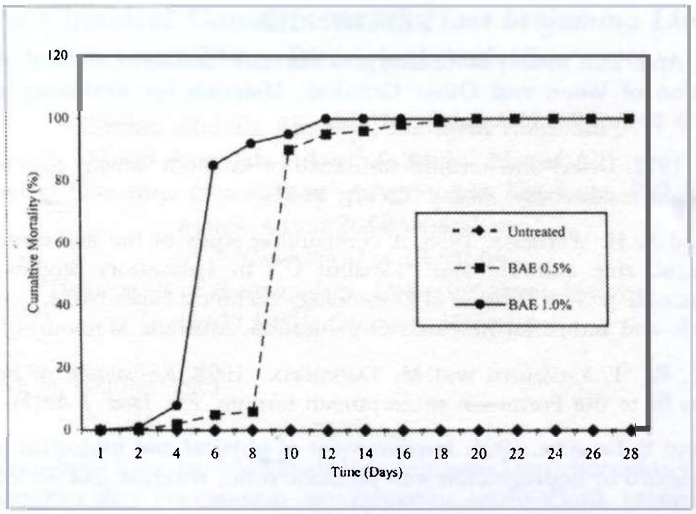

Fig. 2: Cumulative mortality of termites when placed together with treated and untreated particleboard blocks in test bottles for up to 28 days

days of exposure, 16% of the termites died. However, in the bottles containing untreated MUF-bonded particleboard (*Fig. 2*), untreated LVL and solid wood (Table 1), all termites survived after the test period. The results indicate that the PF resin was more toxic to termites than MUF and UF resins. Kajita and Imamura (1993) also reported that the mortality rate for termites fed on phenolic resin treated particleboard gradually increased as the test period continued. The effect was due to the inability of termites to digest the materials.

CONCLUSION

Incorporation of preservatives in oriented strand board, particleboard and laminated veneer lumber provides better resistance against termite attack when compared to the untreated composites. Treatment of PF-bonded OSB with at least 2% of CCA or 1.5% BAE (w/w oven dried flakes) will reduce the damage of termite attack by about 80%. The resistance of UF-bonded particleboards against termite attack increased by 77% when they were treated with 1% BAE. Among the untreated rubberwood composite products, PF-bonded OSB had the highest resistance against termite attack. UF-bonded LVL was more resistant compared to MUF-bonded particleboards. After 4 weeks, all termites which were placed in containers that contained either CCA or boron-treated blocks died. About 16.2% termites survived in containers containing untreated PF-bonded OSB. None of the termites died when they were placed together with MUF-bonded particleboards, UF-bonded LVL and solid rubberwood.

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The Chemical Constituents of *Ficus benjamina* Linn. and Their Biological Activities

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ABSTRAK

Daun-daun, kulit kayu dan buah-buah *Ficus benjamina* Linn. didedahkan kepada pengekstrakan dan pengasingan menggunakan teknik-teknik kromatografik untuk menghasilkan enam sebatian (asid sinamik, laktosa, "naringenin", "quercetin", asid kafeik dan "stigmasterol". Struktur-struktur sebatian tersebut ditentukan oleh teknik-teknik spektroskopik dan perbandingan dengan data yang diterbitkan. Sebatian-sebatian tersebut ditutupi untuk aktiviti antimikrobia ke atas dua spesies bakteria (*Bacillus cereus* dan *Pseudomonas aeruginosa*) dan aktiviti sitotoksik terhadap garis sel "T-lymphoblastic leukemic (CEM-SS). Asid kafeik menunjukkan aktiviti sitotoksik yang kuat dengan nilai IC sebanyak 25 mg/mL.

ABSTRACT

The leaves, bark and fruits of *Ficus benjamina* Linn. were subjected to extraction and isolation using chromatographic techniques to yield six compounds (cinnamic acid, lactose, naringenin, quercetin, caffeic acid and stigmasterol). The structures of the compounds were determined by spectroscopic techniques and by comparison with published data. The compounds were screened for antimicrobial activity against two species of bacteria (*Bacillus cereus* and *Pseudomonas aeruginosa*) and cytotoxic activity against T-lymphoblastic leukemic (CEM-SS) cell line. Caffeic acid exhibited strong cytotoxic activity with IC_{50} value of 25 mg/mL.

Keywords: *Ficus benjamina*, naringenin, quercetin, antimicrobial, cytotoxicity

INTRODUCTION

Ficus benjamina Linn. belonged to the family Moraceae (Burkill 1966). It is a medium-sized tree with several spreading branches from the base. It is widely distributed in the tropics (Baily 1963). The leaves are 2-5 cm wide and the bark is pale brown or greyish brown. The plant is known locally as "beringin, waringin and jejawi" (Holttum 1969). It is traditionally used as a stomachic, hypotensive and anti-dysentery agent (Trivedi *et al.* 1969). Previous studies on *Ficus* species revealed the presence of several compounds such as alkaloids (Beat *et al* 1990), triterpenes (Mohammad *et al.* 1991), ascorbic acid. (Ikhlas *et*

al. 1993) and flavonoids (Ilyas and Ilyas 1990). This paper reports on the isolation and identification of six compounds from the plant and their antimicrobial and cytotoxic activities. There has been no previous report on the chemical constituents and biological activity of *Ficus benjamina.*

GENERAL EXPERIMENTAL

Melting points were determined on a Kofler hot-stage and were uncorrected. IR spectra were recorded on a Perkin-Elmer FTIR 1725X. UV spectra were recorded on a Shimadzu UV-2100 spectrophotometer ^{1}H and ^{13}C NMR spectra were recorded on JOEL 400 MH_z spectrometer using TMS as internal standard in CD_3COCD_3 and DMSO. Mass spectra were obtained using a Finnigan Mat Model SSQ 710 spectrometer.

Plant Material

The leaves, bark and fruits of *Ficus benjamina* were collected from Universiti Putra Malaysia campus in February 1999 and a voucher specimen (10448) was deposited at the Herbarium of the Department of Biology, UPM.

Extraction and Isolation of Compounds

Air dried leaves, bark and fruits of *Ficus benjamina* were extracted successively with petroleum ether, chloroform and methanol. The extracts were concentrated under vacuum. The chloroform extract of the leaves (48.0 grams) was subjected to column chromatography using gradient solvent mixtures; 100% petroleum ether, petroleum ether/chloroform (2:5), chloroform/methanol (8:3) and 100% methanol to yield 15 fractions. Fractions 8 and 9 were further rechromatographed with the chloroform/methanol (8:3) to yield compound (I) as a white powder. Rechromatographing of fractions 10-13 yielded compound (II) as a white powder.

The methanol extract (164.3 grams) was redissolved in 95% aqueous methanol (500 mL) and then reextracted with n-hexane (500 mL). The methanol layer was concentrated, suspended in water (1 L) and then reextracted twice with 500 mL of chloroform, ethyl acetate and n-butanol successively. The n-butanol extract was subjected to column chromatography eluted with 100% chloroform with an increasing amount of methanol to give 18 fractions. Fractions 3-9 were recombined and chromatographed using chloroform/ methanol (1:1) gradient of increasing polarity followed by recrystallisation from methanol to yield compound (III). Fractions 10-18 were also recombined and eluted with chloroform/methanol (5:2) to yield a yellow powder which was recrystallised from methanol to afford compound (IV).

A portion of the n-butanol fraction of the methanol extracts (8.0 g) of the fruits of *Ficus benjamina* was eluted with 100% chloroform, chloroform/methanol (7:3) and 100% methanol to give 16 fractions. Fractions 3-10 were concentrated and analysed by TLC to give white solid after washing with ethanol repeatedly

and recrystallised from ethanol to give compound (V). However, the chloroform extract of the bark (10.0 g) yielded only one compound (VI).

Antimicrobial Assay

The microorganisms were obtained from the culture collection of the Department of Biotechnology, UPM. The stock cultures were grown on potato dextrose agar (PDA) for 24 h at 28°C at which time the cells were harvested by centrifugation (4°C, 2000 rpm, 3 min.). The cells were washed and suspended in sterile 0.9% saline to give a final concentration of 10^5-10^6 CFU/mL using a haemocytometer (Berdy 1985; Bergeys 1957). The microbial strains used were: *Bacillus cereus* NRRLUI-1447, *Pseudomonas aeruginosa* UI-60690; *Aspergillus ochraceus* NRRL 398, *Candida lipolytica* ATCC 2075, *Sacchromyces cereviseae* NRRL 2034 and *Sacchromyces alipolytica.*

Antimicrobial activity of the isolated compounds were tested using disc diffusion method according to Bauer *et al.* (1966). The discs were prepared by impregnating them in ethanolic solution of each sample (10 mg/mL). They were then evenly spaced out on the agar surface previously inoculated with the suspension of each microorganism to be tested. Standard discs of nystatin (50 g/discs) and streptomycin sulphate (25 g/discs) were used as positive controls. The plates were incubated at 37°C for 24 h and the antimicrobial activity was recorded by measuring the diameter of the clear inhibition zones around each disc.

Cytotoxity Test

The cytotoxic effect of the compounds was evaluated on (CEM-SS) T-lymphoblastic leukemic cell line which was obtained from the National Cancer Institute, Maryland, USA and maintained as previously described (Weilow *et al.* 1989). Cell viability was determined by using the microtitration assay (Ali *et al.* 1998). Cytotoxicity was recorded as the 50% inhibition concentration (IC_{50}) with reference to the untreated positive control cells (Ali *et al.* 1997).

RESULTS AND DISCUSSION

Extraction of the leaves, bark and fruits of *Ficus benjamina* followed by extensive column chromatography resulted in the isolation of cinnamic acid, lactose, naringenin, quercetin, caffeic acid and stigmasterol.

Compound (I) was obtained as white powder (24.9 mg) from chloroform extract of the leaves of *Ficus benjamina* with melting point 128-130°C (Buckingham 1994, 130-131°C). The aromatic regions in ^{1}H-NMR spectrum indicated the presence of five protons at d 7.83, 7.56, 7.42, 7.36, 7.24 each was assigned to proton in positions-3, 4, 5, 6 and 2 respectively. The other two signals which appeared at d 6.49 and 6.43 indicated the presence of two protons at C-7 and C-8. The presence of seven signals in the ^{13}C-NMR spectrum indicated the presence of nine carbons in the molecule which include seven methine carbons at 117.8, 147.5, 128.8 (×2), 134.5 and 129.4(×2) ppm assigned to C-2, C-3,

C-5 and C-6 (overlapped), C-4, C-7 and C-8 (overlapped) respectively. The two quartenary carbons at 131.2 and 173.2 ppm were assigned to C-1 and C-9. The mass spectrum indicated the presence of a molecular ion peak at m/z 148 which corresponded to the molecular formula $C_9H_8O_2$ with a base peak at m/z 66. Loss of one molecule of water was indicated by the fragment at m/z 130. Comparison of these spectral data with those reported previously (Byung *et al.* 1996) supported that Compound (1) is cinnamic acid.

Lactose (II) was isolated as white powder with melting point 229-230°C (Tadasu *et al.* 1985, 230-232°C). The chemical test and the spectral data obtained for these compounds were in agreement with published data (William 1987).

Naringenin (III) was obtained from the n-butanol fraction of the methanol extract of the leaves of *Ficus benjamina* as white powder with melting point 249-252°C (Buckingham 1994, 250-251°C). The ^{1}H-NMR spectrum indicated the presence of two hydroxyl groups which appeared as broad singlets at δ 9.73 and δ 8.63 assigned to 4′-OH and 7-OH respectively. The other hydroxyl group appeared as a sharp singlet at δ 12.18 indicative of a chelated hydroxyl proton at C-5. A doublet of a doublet at d 7.41 (J=1.9 H_z) was assigned to the two equivalent protons at H-3′ and H-5′ respectively. The other two equivalent protons which also appeared as a doublet of a doublet at δ 6.92 (J=1.9 H_z) was assigned to protons at H-2′ and H-6′. Another two protons which appeared as a pair of doublets at δ 5.96 (J=1.9 H_z) were assigned to the two protons at C-6 and C-8. A doublet at δ 5.42 (J=2.9 H_z) was assigned to proton at C-2 which is coupled to the methylene protons at H-3. The signal for H-3a appeared at d 2.74 as a doublet of a doublet with coupling constant values of 3.2 H_z ($J\,H_{3a\text{-}2}$) and 3.0 H_z ($J\,H_{3a\text{-}3b}$). The H-3b proton gave a signal at δ 3.27 with coupling constant values of 4.2 H_z ($J\,H_{3b\text{-}2}$) and 2.9 H_z ($J\,H_{3b\text{-}3a}$). The ^{13}C-NMR spectrum assignments were made by DEPT experiment and also by comparison with literature values (Eberhaed and Wolfgang 1987; Agrawal 1989). The spectrum showed the presence of fifteen carbons consisting of seven quartenary carbons at 197.2, 167.3, 165.5, 164.6, 158.7, 130.8 and 103.2 ppm which were assigned to C-4, C-7, C-5, C-9, C-4′, C-1′ and C-10 respectively. The seven methine carbons were at 129.0 (×2), 116.2 (×2), 95.8, 96.8 and 79.9 ppm assigned to C-3′ & C-5′, C-2′ & C-6′, C-6, C-8 and C-2 respectively. The methylene carbon for C-3 appeared at 43.5. Mass spectrum gave a molecular ion at 272. This is consistent with the presence of three hydroxyl groups and a molecular formula of $C_{15}H_{12}O_5$. Based on the spectral data obtained and compared with published data (Barakat *et al.* 1999), Compound III concluded to be naringenin.

Quercetin (3,5,7,3′,4′-pentahydroxyflavone) Compound (IV) was isolated from the n-butanol fraction of the methanol extract of the leaves of *Ficus benjamina* as yellow needles with melting point 296-298°C (Uphof 1968, 298-300°C). The ^{1}H-NMR spectrum of compound (IV) showed peaks at δ 9.36. These are characteristics of a 3′ and 4′-disubstituted B-ring of flavonol (Mabry *et al.* 1970; Banerji *et al.* 1969). The two broad singlets at δ 10.86 and 9.66 may be attributed to the two hydroxyl groups at C-7 and C-3 respectively, whereas

the hydroxyl group at position–5 appeared at very low field as a sharp singlet at δ 12.52 due to chelation to the carbonyl group. The aromatic region in the ^{1}H-NMR spectrum showed the presence of five protons each. A pair of doublets at δ 6.48 and 6.22 each with coupling constant 2.2 H_z due to *meta* coupling were assigned to protons H-8 and H-6, respectively. Another doublet was observed at δ 7.71 with coupling constant 2.2 H_z assigned to H-2′. Proton at H-6′ occurred as doublet of a doublet at δ 7.57 with coupling constant 2.2 H_z. The doublet at d 6.95 with coupling constant 8.5 H_z was assigned to proton at H-5′. The ^{13}C-NMR spectrum indicated the presence of fifteen carbon atoms including ten quartenary carbons at 146.9, 135.8, 175.9, 156.2, 163.9, 160.8, 103.1, 122.1, 145.1 and 147.8 ppm assigned to C-2, C-3, C-4, C-5, C-7, C-9, C-10, C-1′, C-3′ and C-4′ respectively. The five methine carbons occurred at 98.3, 93.4, 115.1, 115.7 and 120.1 ppm assigned to C-6, C-8, C-2′, C-5′ and C-6′ respectively. This was supported by the results obtained from DEPT experiment. The mass spectrum gave a molecular ion peak at m/z 302 which is consistent with the molecular formula of $C_{15}H_{10}O_7$. The structural assignment of this compound was supported by m.p., IR, UV, ^{1}H, ^{13}C-NMR, DEPT and comparison with published data (Buckles 1962).

Caffeic acid (V) was isolated as light yellow powder (25.5 mg), with melting point 195-198°C (Kiritsakis 1998, 200°C). The ^{1}H-NMR spectrum revealed the presence of two doublets at δ 6.80 and 6.95 each with coupling constants of 8.1 H_z assigned to the two protons at C-5 and C-6 respectively. Another aromatic proton on positin H-2 occurred as sharp singlet at δ 7.05. The two methine protons at positions H-7 and H-8 appeared as two doublets at δ 7.57 (J=15.8 H_z) and 6.21 (J=15.8 H_z), whereas the broad singlet peaks at δ 4.95 indicated the presence of two hydroxyl groups in positions 3 and 4.

The ^{13}C-NMR spectrum showed the presence of nine carbons in the spectrum in which five were methine carbons (three aromatic carbons and two aliphatic carbons) at 117.4, 116.0, 123.8, 148.0 and 116.4 ppm assigned to C-2, C-5, C-6, C-7 and C-8 respectively. The four quartenary carbons at 128.7, 147.6, 150.3 and 172.0 ppm were assigned to carbon signals at positions C-1, C-3, C-4 and C-9 respectively. The mass spectrum indicated the presence of a molecular ion as the base peak at m/z 180 which corresponds to the molecular formula $C_9H_8O_4$. Loss of one hydroxyl group from the molecular ion gave a peak at m/z 163 and subsequent loss of another water molecule from this fragment ion gave a fragment at m/z 145. Thus the compound was identified as caffeic acid (3, 4-dihydroxy cinnamic acid).

Stigmasterol (VI) was obtained from chloroform extract of the bark of *Ficus benjamina* as white powder with melting point 166-167°C (Schwartz and Wall 1995, 166-168°C). Based on the spectral data and on comparison with literature values (Cechinel *et al.* 1998), the compound was identified as stigmasterol.

Antibacterial screening on the compounds showed that quercetin and naringenin exhibited the stronger antibacterial activity. Cinnamic acid and lactose showed strong activity against the Gram-negative and moderate activity against the Gram-positive bacteria. Caffeic acid and stigmasterol yield weak

activity against the two species of bacteria. However, all the compounds did not show inhibitory activity against the four species of fungi. They showed negative cytotoxicity effect, except for caffeic acid, which exhibited strong cytotoxic activity against T-lymphoblastic leukemic (CEM-SS) cell line with an IC_{50} value of 25 mg/mL (Table 1 and *Fig. 1*).

The cytotoxic effect of caffeic acid at the inhibition concentration (IC_{50}) value as compared with control is shown in *Fig. 2* in which the cells became granulated and fragmented.

TABLE 1
Antimicrobial activity of compounds isolated (concentration 100 µg/ml, methanol) from *Ficus benjamina*

compound	bacteria		fungi			
	B. cereus	*P. aeruginosa*	*A. ochraceous*	*C. lipolytica*	*S. cereviseae*	*S. lipolytica*
cinnamic acid	++	++	-	-	-	-
lactose	+	+	-	-	-	-
naringenin	+++	+++	-	-	-	-
quercetin	+++	+++	-	-	-	-
caffeic acid	+	+	-	-	-	-
stigmasterol	++	++	-	-	-	-

B. = *Bacillus* *P.* = *Pseudomonas* *A.* = *Aspergillus* *C.* = *Candida*
S. = *Sacchromyces* - no inhibition (0 mm) + weak inhibition (1-9 mm)
++ medium inhibition (10-14 mm) +++ strong inhibition (15-19 mm)

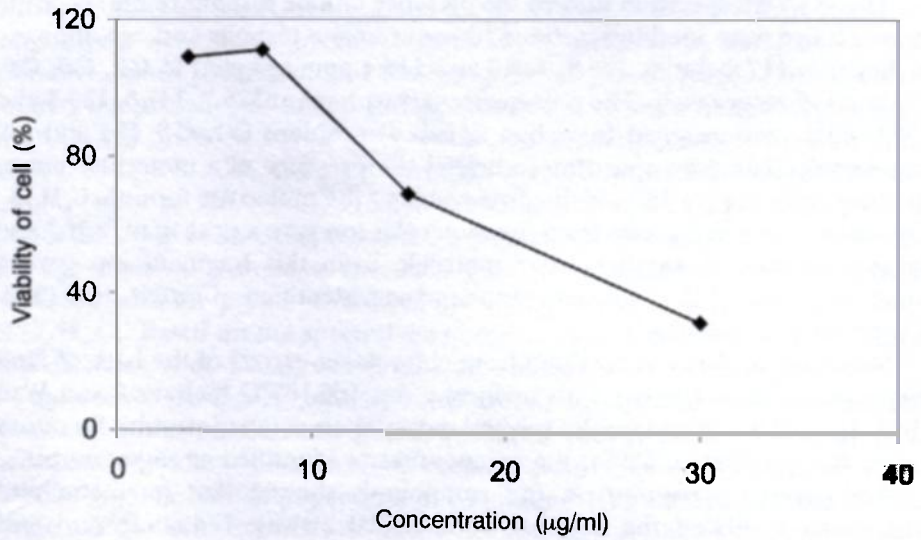
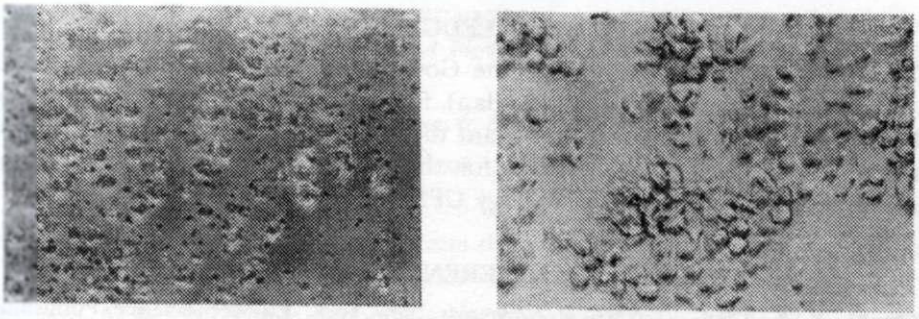
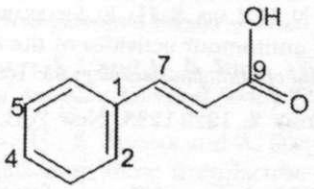


Fig. 1. Percentage viability of (CEM-SS) cells after being treated with different concentrations of caffeic acid

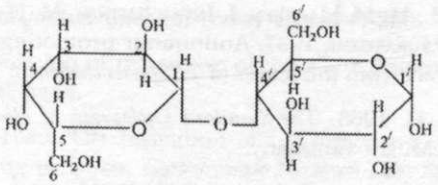


(a) (b)

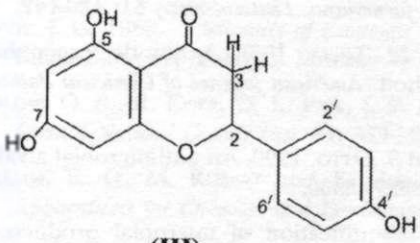
Fig. 2: Morphology of (CEM-SS) cells line treated for 72 hours (a) 15 (g/ml of caffeic acid (b) control



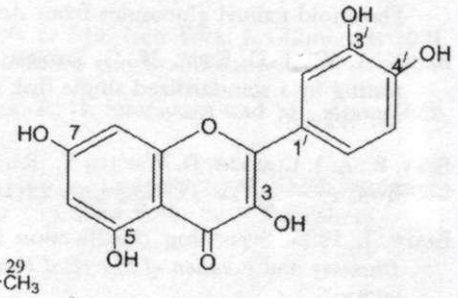
(I)



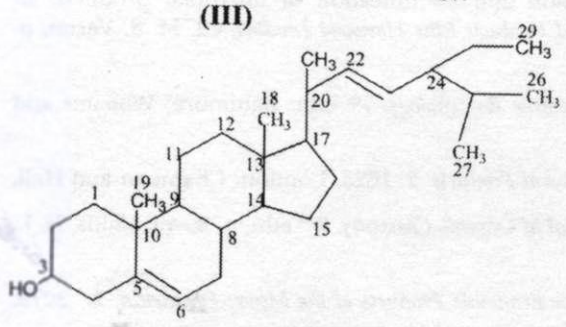
(II)



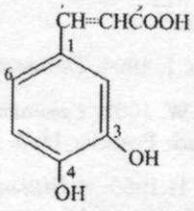
(III)



(IV)



(VI)



(V)

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Measurement of Thermal Diffusivity, Optical Transmission and Optical Absorption Peaks of Laser Dyes R6G Doped in Poly (Methylmethacrylate) Using Photoacoustic Technique and Fibre Optics Spectrophotometer

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ABSTRAK

Dengan menggunakan teknik spektroskopi fotoakustik (PA), pengukuran peresapan terma Rhodamine 6G (R6G) didop dalam matriks pepejal poli(metilmetalkrilat) (PMMA) pada pelbagai kepekatan dilaporkan. Nilai peresapan terma didapati bertambah dengan kepekatan 'dye' disebabkan pertambahan penyerapan optik molekul 'dye' dalam matriks polimer. Spektrofotometer serabut optik, uv-sinar nampak juga digunakan untuk mengukur spektrum optik sampel. Hasil kajian menunjukkan bahawa transmisi penggal dalam kawasan merah (500-600) nm berganjak kepada jarak gelombang yang lebih tinggi bila kepekatan 'dye' bertambah. Transmisi maksimum (83%) didapati berlaku pada sampel PMMA yang didopkan dengan kepekatan 'dye' R6G yang paling rendah, iaitu 3.1×10^{-4} mol/*l*.

ABSTRACT

Using the photoacoustic (PA) spectroscopic technique, we report the thermal diffusivity measurement of the Rhodamine 6G (R6G) doped at different concentrations in solid matrix poly(methylmethacrylate) (PMMA). The value of the thermal diffusivity was found increasing with dye concentration due to the increase of optical absorption of dye molecules in the polymer matrices. A uv-visible fibre optics spectrophotometer was also used to measure the optical spectrum of the samples. The result shows that the transmission cut off in a red region (500-600) nm shifted to the higher wavelength when the dye concentration increased. The maximum transmission (83%) was observed for the PMMA sample doped with the lowest concentration of dye, i.e. 3.1×10^{-4} mol/*l* of R6G.

Keywords: Thermal diffusivity, optical transmission, absorption peak, photoacoustic, Rhodamine 6G, and poly (methylmethacrylate)

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INTRODUCTION

Organic dyes have been used for many years in both lasers and optically pumped amplifiers. A solid-state dye laser offers attractive qualities such as high quantum efficiency, a large choice of pump source, and a broad emission bandwidth. They are of continuing interest because of their capacity, with different dyes, to cover the spectrum from the ultraviolet to the infrared (Amaud *et al.* 1996; Mark and King 1995).

Solid-state dye lasers were first demonstrated as early as 1967 and have recently reached the commercial market. Numerous research works have been done for dyes doped in several different solid matrices including polymers, solgel, ormosil and xerogel glasses. Efficient solid-state dye lasers were recently obtained using polymeric matrices. One of the main concerns in recent research for particular laser dyes and solid hosts is to provide a high laser damage threshold and a long lifetime against photodegradation. The photostability of some organic laser dyes improves when molecules are trapped into solid matrices (William *et al.* 1995; Cazeca *et al.* 1997). Poly (methyl methacrylate) (PMMA) is a well-known polymeric matrix that is the most commonly used for solid-state dye lasers. It has a good optical transparency and a lower laser damage threshold. Rhodamine 6G (R6G) is the most widely employed laser dye in recent years. R6G is a xanthene derivative with an optical absorption peak in ethanol at 530 nm and a fluorescence peak at 556 nm. It has a high fluorescence quantum yield (~ 95%), a low intersystem crossing rate and low exited-state absorption. These properties make it a highly efficient dye for both pulsed and continuous-wave laser actions (Mark *et al.* 1995).

Thermal diffusivity is a unique property for each material and the diffusion of heat in the material is an important transport property that has a major concern for the manufacturing technology. In this paper, we report the thermal diffusivity measurement using the photoacoustic technique for poly(methyl methacrylate)(PMMA) doped with Rhodamine 6G (R6G) at different concentration levels. We also investigated the optical transmittance and absorbance spectrum of the sample using a uv-visible fibre optics spectrophotometer.

MATERIALS AND METHODS

The PA technique is reported to be a versatile tool for optical and thermal characterization of a variety of solid samples. The PA technique makes use of the detection of acoustic waves generated by a modulated optical irradiation when it interacts with a sample kept inside a closed cavity. According to Rosencwaig-Gersho theory (Rosencwaig and Gersho 1976), for a thermally thick sample, the complex amplitude of the pressure variation inside the cavity is given by

$$Q = \frac{-i\beta\mu^2\gamma P_0 I_0}{4\sqrt{2}T_0 l' a' k} \quad (1)$$

where β and μ are the optical absorption coefficient and the thermal diffusion length of the sample, respectively; γ is the ratio of heat capacities of air; P_o and T_o are the ambient pressure and temperature, respectively. I_o is the incident light intensity and l' is the length of the gas column inside the cavity; a' and k are the thermal diffusion coefficient of the gas inside the cavity and the thermal conductivity of the sample, respectively. We have selected the chopping frequency and the sample thickness in such a way that the sample under investigation is thermally thick in all the experiments. From the above equation, it is clear that under identical experimental conditions, the PA signal amplitude is directly proportional to the optical absorption coefficient of the sample (Nibu *et al.* 1999).

The absorption characteristic of materials under normal circumstances can be represented by the equation:

$$A = \log_{10}\left(\frac{I_0}{I}\right) \quad (2)$$

where I_o is the intensity of the incident radiation and I is the intensity of the radiation transmitted through the sample. The quantity $\log_{10}(I_o/I)$, which is measured directly by the uv-visible spectrophotometers, is called the absorbance (A) or the optical absorption. Transmission (T) is a measurement of how much light is transmitted through a sample. Common applications include the measurement of transmission and absorption of light through the optical filters, optical coatings, and other optical elements such as lenses and fibers (Norman and Mckellar 1980).

PMMA and R6G (BDH Chemicals) were used as received. Two grams of PMMA were dissolved in 25 ml of chloroform. Solutions of five different R6G dye concentrations (3.1×10^{-4} mol/l, 6.3×10^{-4} mol/l, 1.9×10^{-3} mol/l, 3.1×10^{-3} mol/l and 6.3×10^{-3} mol/l) were prepared. Each solution was vigorously stirred to afford a viscous mixture that was then poured into a petry dish and kept in the dark at room temperature for the solvent to be evaporated. After 24 h, the completely solid samples were cut into pellet-shape pieces, 10 mm in diameter and (0.47-0.50) mm in thickness for further measurements.

The experimental set up used for the present study is shown in *Fig. 1.* The diode laser (B & W TEK) beam, which after being mechanically chopped by an optical chopper (SR540), was focused onto the sample kept inside a non-resonant PA cell. The photoacoustic signal generated in the closed cavity was detected using a sensitive microphone (Cirkit product). The PA signal was then processed using a lock-in amplifier (SR530). The photostability of the samples was first investigated at fixed chopping frequency (i.e. 15 Hz) for 5

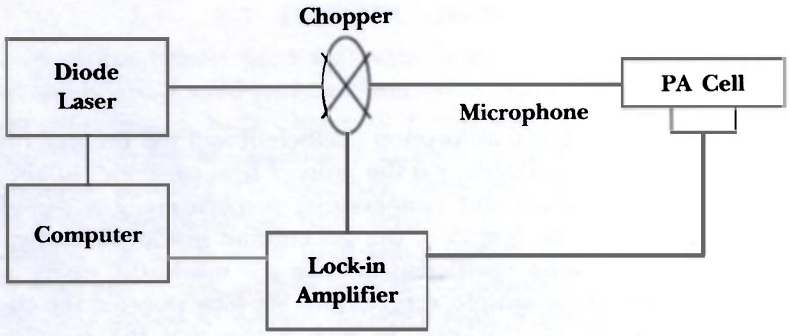

Fig. 1. Schematic diagram of the experimental set-up

different laser powers; 300 mW, 290 mW, 280 mW, 270 mW and 260 mW. After laser irradiation for 1 h, the photoacoustic signal for each PMMA sample was measured for the chopping frequency in the range of (10-150) Hz.

A uv-visible spectrophotometer is a device for measuring spectra; it enables quantitative measurement of light intensities to be made at different wavelengths. In the present study, a uv-visible fibre optics spectrophotometer (OCEAN Optics, S2000) was used to measure the transmission and absorbance spectra of each sample at different dye concentrations. It consists of a uv-visible light source, optical fibre and diode arrays detector, which was interfaced to a personal computer. The acquired data is then displayed and analysed by using the OOIBase software provided by the system.

RESULTS AND DISCUSSION

First, we measured the PA signals as a function of time for five samples with different dye concentrations at a fixed chopping frequency (i.e. 15 Hz). We also measured the variation of PA signals with time for each sample with five different beam powers. The PA signal observed does not significantly change with the increasing time. This indicates that the R6G-doped PMMA samples are stable and do not show any noticeable photodegradation after being exposed to laser irradiation. Thus the value of the PA signal is purely due to the contribution of the R6G-doped PMMA matrix. Therefore this observation confirmed that R6G is a potential candidate for relatively stable laser action in the host used. These properties make it a highly photostable and efficient dye laser medium for both pulsed and continuous-wave laser actions (Mark *et al.* 1995).

A plot of ln (PA Signal) as a function of ln $(f)^{1/2}$ for each sample is shown in *Fig. 2.* The detected photoacoustic signal for the doped sample exhibits $f^{-0.88}$, $f^{-0.93}$, $f^{-0.97}$, $f^{-1.01}$ and $f^{-1.07}$ modulation frequency dependence on increasing dye concentration. This confirmed that the samples used in this study were thermally thick samples.

Since our samples are thermally thick (470 - 500) μm, the thermal diffusivity of each PMMA sample was obtained by fitting the experimental data to the

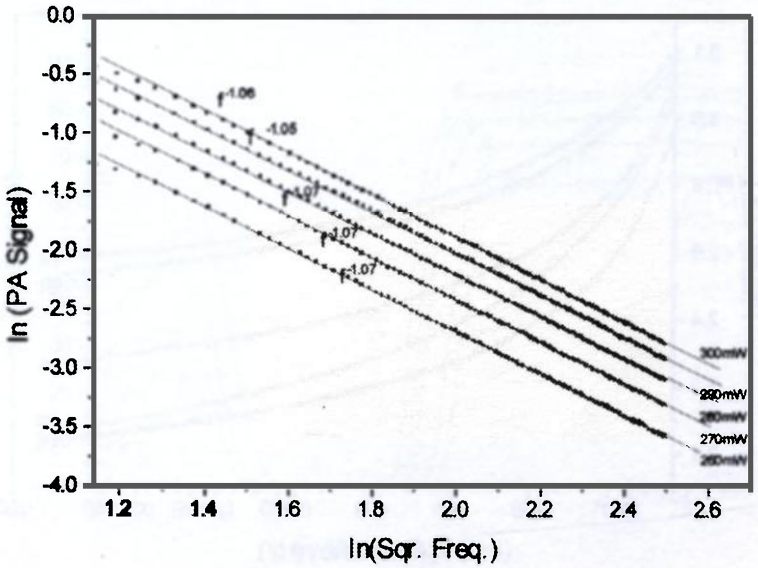

Fig. 2. Plot of ln (PA Signal) as a function of ln $(f)^{1/2}$ at different laser power for sample at dye concentration $6.3x10^{-3}$ mol/l

phase-frequency dependent relation given by Silva *et al.* (1998). This relationship was only considered for thermally thick samples, which is applied to the present condition, i.e.

$$\Phi = \Phi_o + \arctan \{ L_s [(\pi / \alpha_s) f]^{1/2} - 1 \}^{-1} \quad (3)$$

where Φ_o is the initial phase, α_s is the thermal diffusivity, L_s is the sample thickness and f is the chopping frequency. *Fig. 3* shows the phase signal as a function of chopping frequency for different concentrations of R6G doped PMMA samples. The thermal diffusivity values obtained for these samples at different dye concentrations are 1.48×10^{-3} cm^2/s, 1.58×10^{-3} cm^2/s, 1.83×10^{-3} cm^2/s 2.05×10^{-3} cm^2/s and 2.17×10^{-3} cm^2/s, respectively. A plot of the thermal diffusivity as a function of dye concentration is shown in *Fig. 4*. The thermal diffusivity appears to increase with increasing dye concentration in PMMA samples. The plot can be described by an empirical relationship as:

$$\alpha = (1.38 \times 10^{-3}) + 0.298C - 27.34C^2 \quad (4)$$

where C is the dye concentration in mol/*l*. The thermal diffusivity value for an undoped PMMA sample could be estimated by extrapolating the curve to C = 0, and the value obtained for the undoped PMMA samples was ~1.38×10^{-3} cm^2/s. This is about 17% higher than the value calculated from the value of k, ρ and C_p of pure PMMA (Costela *et al.* 1995).

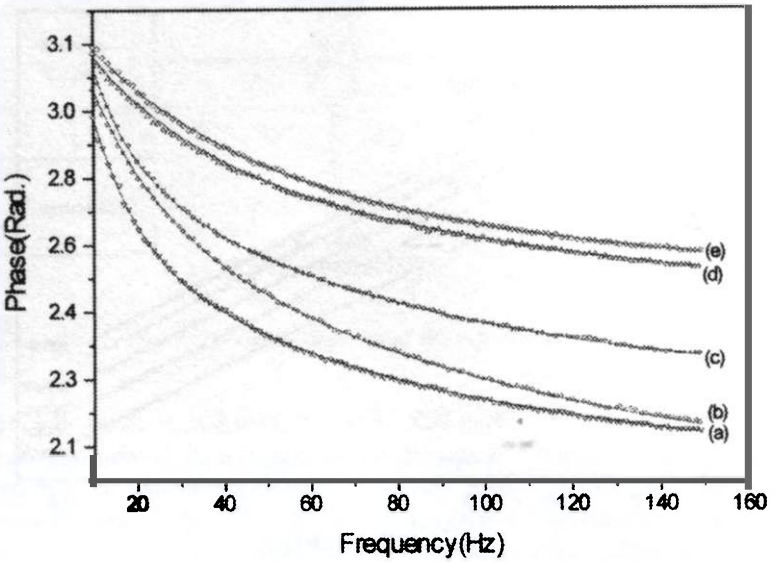

Fig. 3. Phase signal versus chopping frequency for R6G doped PMMA samples at different dye concentrations: (a) 3.1 × 10^{-4} mol/l; (b) 6.3 × 10^{-4} mol/l; (c)1.9 × 10^{-3} mol/l (d) 3.1 × 10^{-3} mol/l (e) 6.3 × 10^{-3} mol/l. The solid curves represent the fitting of the experimental data to Eq.(3)

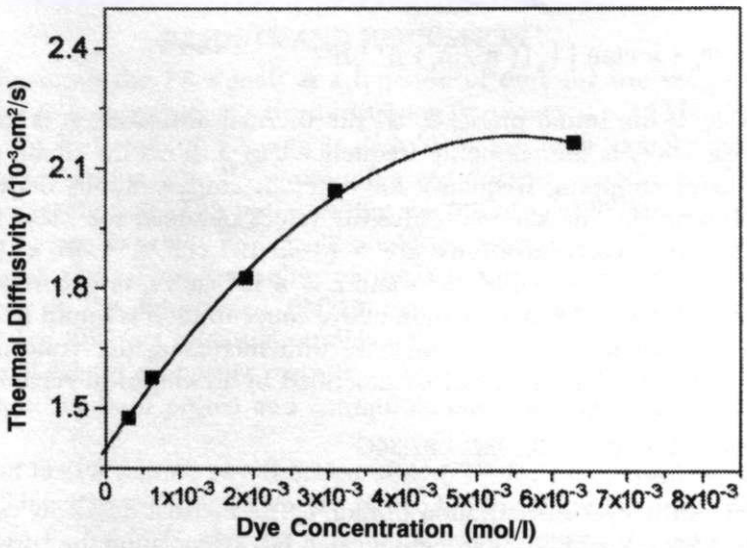

Fig. 4. Thermal diffusivity versus the concentration of dye R6G doped in PMMA samples

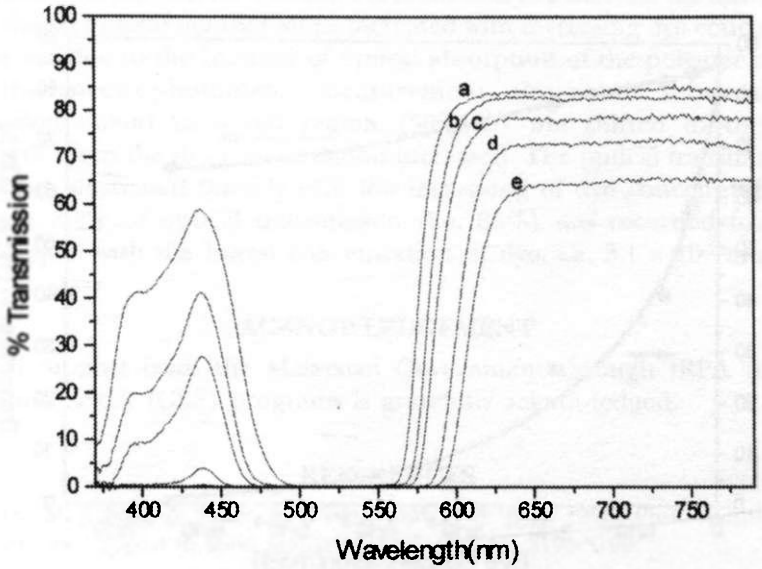

Fig. 5. The transmission spectra of the R6G doped PMMA samples at different concentrations: (a) $3.1x10^{-4}$ mol/l; (b) $6.3x10^{-4}$ mol/l; (c)$1.9x10^{-3}$ mol/l (d) $3.1x10^{-3}$ mol/l (e) $6.3x10^{-3}$ mol/l

Fig. 5 shows the measured transmittance spectra of the samples at different dye concentrations. For $\lambda > 650$ nm, the measured optical transmissions were 83%, 82%, 77%, 73% and 65%, i.e. they decrease with increasing dye concentration. The cut-off transmission occurred in the range of (500-550) nm and became broader when the concentration of dye increases. This material could be a good filter for laser beams, particularly for Ar^+ ion laser with the output of 514 nm. The transmission peak seems to occur at wavelength 438 nm. This peak height decreased with increasing dye concentration as shown in *Fig. 6*. *Fig. 6* also shows that for $\lambda > 650$ nm, the decreasing of transmission is linear with the dye concentration. The absorbance spectra for R6G–doped PMMA samples at different dye concentrations are shown in *Fig. 7*. The plateau at the top of curves at dye concentrations 3.1×10^{-3} mol/l and 6.3×10^{-3} mol/l is due to the saturation of the spectrophotometer signal, resulting from the higher concentration of dye doped in the samples (Cazeca *et al.* 1997). Obviously, the observed maximum absorbance for R6G-doped in PMMA samples occurred at the wavelength centered around 526 nm.

CONCLUSION

The results presented in this paper lead to two main conclusions. First, the photoacoustic technique is efficient in measuring the thermal diffusivity of polymer samples. Thus it has been used for measuring thermal diffusivity of

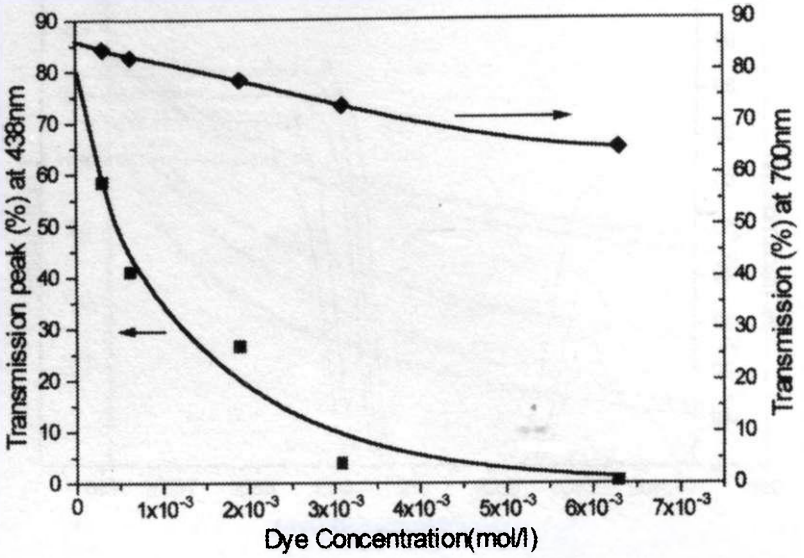

Fig. 6. Optical transmission of PMMA samples doped with different concentration of R6G

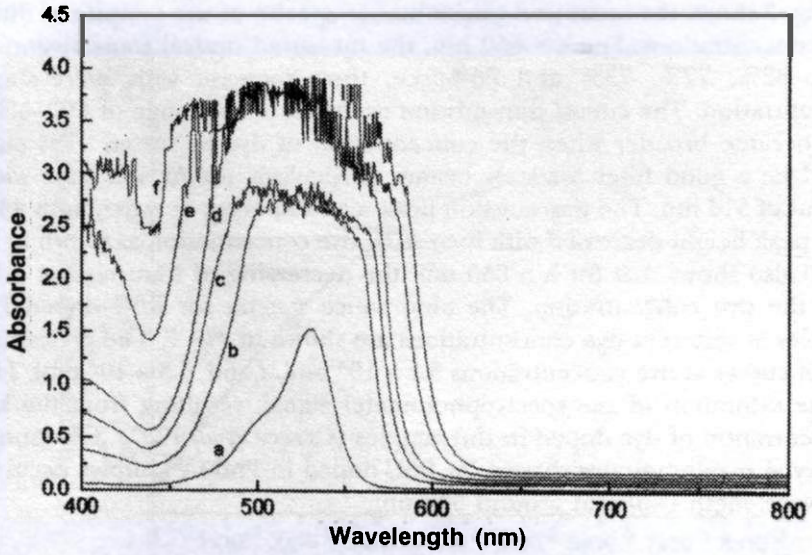

Fig. 7 Absorbance spectra of the R6G doped PMMA sample at different dye concentrations: (a) pure R6G in ethanol (b) $3.1x10^{-4}$ mol/l ; (c) $6.3x10^{-4}$ mol/l; (d) $1.9x10^{-3}$ mol/l ; (e) $3.1x10^{-3}$ mol/l; (f) $6.3x10^{-3}$ mol/l

R6G in solid matrix PMMA. We also observed that the thermal diffusivity values of R6G doped in solid matrix PMMA increased with increasing dye concentration and this was due to the increase of optical absorption of the polymer matrices.

In the spectrophotometer measurement, the result shows that the transmission cut-off in a red region (500-600) nm shifted to the higher wavelength when the dye concentration increased. The optical transmission for $\lambda > 650$ nm decreased linearly with the increasing of dye concentration. The maximum value of optical transmission (i.e. 83%) was recorded for PMMA sample doped with the lowest concentration of dye, i.e. 3.1×10^{-4} mol/*l*.

ACKNOWLEDGEMENT

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Numerous methods have been developed for the analysis of organophosphorus pesticides. Lee *et al.* (1991) demonstrated the use of acetonitrile for the extraction of 7 OP pesticides in vegetables and fruits and cleaned up on reverse phase solid phase extraction (SPE). Final determination was carried out by gas chromatography (GC) using a flame photometric detector (FPD). Sasaki *et al.* (1987) reported a simple and efficient cleanup method for GC determination of 23 OP pesticides in crops including onion using GC-FPD. The sample was extracted with acetone and benzene. A multiresidue method for the quantitative determination of OP pesticides in both fatty and non-fatty foods was developed by Blaha *et al.* (1985). The sample was extracted with acetone, methylene chloride-hexane (1:9) and methylene chloride-acetone (3:1). Fatty food extracts are cleaned up on gel permeation chromatography (GPC). Leoni *et al.* (1992) reported a method for the determination of 28 OP pesticides in fatty and non-fatty foods. Extraction was carried out using acetone, acetone-water and cleanup on carbon-celite. Holstege *et al.* (1991) described a multiresidue method for the determination of 43 OP insecticides in plant and animal tissues. The OP insecticides were extracted with methanol-dichloromethane (1:9) and cleaned up using automated GPC and silica gel mini columns.

The present method used in the laboratory for the analysis of OP pesticides does not include cleanup procedures prior to GC-FPD determination. As a result, contamination often occurs in the GC inlet and GC column. In order to address this problem, a simple and rapid cleanup method needs to be developed for routine analysis. The SPE method was chosen because less solvent is used, sample clean up time can be reduced and cross-contamination can be eliminated.

Several SPE methods for OP pesticides cleanup in different matrixes have been reported. Bennett *et al.* (1997) described a method using octadecyl (C18) and aminopropyl (NH_2) silica cartridges for the determination of 19 OP pesticides in liquid whole milk. Fillion *et al.* (2000) developed a method for the determination of OP pesticides in fruits and vegetables using octadecyl C18, carbon and aminopropyl cartridges. Cook *et al.* (1999) used C18 SPE as cleanup for the detection of pesticides in spinach, oranges, tomatoes and peaches. A method using silica gel SPE cleanup for OP nematicides determination was described by Cooper *et al.* (1994). Jansson (2000) reported good recoveries for OP pesticides, parathion, trichlorfon and dimethoate in honey using Isolute ENV+. Sheridan *et al.* (1999) used quaternary amine (SAX), PSA and Envi-carb for OP pesticides cleanup prior to gas chromatograph-mass spectrometry (GC/MS) determination. A method using C18 and LC-amino for cleanup of soil extract and GC/MS determination was reported by Mogadati *et al.* (1999). Yamazaki *et al.* (1999) described a method using SAX and PSA for cleanup of chlorpyrifos and methyl parathion in oranges. Gillespie *et al.* (1995) used C18 and alumina cleanup method for OP in vegetable oils and butter fat. An SPE C18 method for cleanup of methyl parathion and methyl paraoxon in milk was reported by Baynes *et al.* (1995).

This paper reports the determination of OP pesticides in vegetables using GC-FPD. Residues were extracted with acetone and dichloromethane. Extracts were cleaned up on solid-phase extraction, SAX/NH_2. The results obtained were compared to the method currently used in the laboratory.

PROCEDURE/METHOD

Chemicals and Reagents

All the chemicals and reagents were of analytical grade. Pesticide standards were obtained from Dr. Ehrenstorter™ , Germany. SPE tubes, Isolute SAX/NH_2 (1 g sorbent in 6 mL tube) were purchased from International Sorbent Technology.

Instrumentation

A Hewlett-Packard Gas Chromatograph 5890 Series II equipped with Flame Photometric Detector was used. GC conditions were : Injector temperature, 260°C; detector (FPD) 250°C; carrier flow (nitrogen) 4 mL/min; oven temperature, 120°C (1.0 min), rate 30°C/min to 150°C, rate 5°C/min to 270°C (10 min); air flow : 80 mL/min; hydrogen flow : 67 mL/min. OP pesticides were analysed on a HP5, 15 m x 0.53 mm x 1.5 mm column.

Procedures

Three types of vegetables: carrot (*Daucus carota*), cucumber (*Cucumis sativa*), and green mustard (*Brassica chinensis*) which represent root, cucurbits and brassica families respectively were selected for this study. Each sample was fortified with eleven pesticide standards at 0.5 ppm. Three replicate fortifications for each matrix type were prepared.

Extraction was carried out based on procedures described by Steinwandter (1985). Fifty g of the sample was homogenised with 100 mL acetone, 75 mL dichloromethane and 15 g sodium chloride in a blender for 3 min. The organic phase was transferred to a beaker and 3 g of sodium sulphate was added. For the routine method, the extract was injected directly into the GC for OP determination without cleanup. For the SPE method, the SAX/NH_2 sorbent was used for cleanup of the OP extract.

The SAX/NH_2 was conditioned with 10 mL of acetone : petroleum ether (1:2). 2 mL of extract was transferred to the SPE tube. The SPE tube was eluted with 10 mL of conditioning solvent at a flow rate of 1 mL/min. The eluate was analysed for OP pesticides using GC-FPD.

Statistical Analysis

A t-test was used to determine the significance of the difference between the current method and the SPE method in terms of recovery. They were considered significantly different when values of t(calculated) > t(critical) at 95% confidence level.

RESULTS AND DISCUSSION

Optimisation of SPE Conditions

The SPE conditions were optimised to obtain good recoveries for OP pesticides. Among the parameters studied were flow rate and solvent polarity. The studies showed that a flow rate of 1 mL/min for the elution was sufficient to recover most of the OP pesticides. The flow rate can be increased to 3 – 4 mL/min without affecting the results.

Development of the solvent system was based on several criteria. Solvents which were hazardous or expensive to dispose of were not evaluated. A volatile solvent system must be used as rapid evaporation of a large solvent volume would be required in sample preparation without causing loss of volatile pesticides. The solvent system must be sufficiently polar to extract most polar pesticides. The final sample extract should have minimum matrix co-extractives.

Mixtures of three solvents were used in this study: acetone, hexane and petroleum ether. A low polarity solvent mixture such as acetone:hexane (5:95) did not sufficiently remove all the OP pesticides. Increase of the solvent mixture polarity improved recovery. It was found that acetone : petroleum ether (1:2) resulted in good recoveries for most of the OP pesticides studied except methamidophos. However, increasing the composition of polar solvent further resulted in low recoveries for some of the pesticides studied. The critical factor observed was that the SPE tube should not be left dry after eluting the samples, since low recoveries may result. Ten mL of eluting solvent was sufficient to condition the SPE tube and to elute the analytes. Increasing the amount of solvent for elution did not improve recovery. The optimal conditions for OP pesticides were found to be as follows : (a) solvent mixture:acetone: petroleum ether (1:2); (b) volume of eluting solvent:10 mL; (c) flow rate of elution:1 mL/min.

Recovery Studies

Eleven OP pesticides were selected for this study. They were methamidophos, dimethoate, diazinon, tolcofos-methyl, fenitrothion, chlorpyrifos, phenthoate, prothiofos, triazofos, cyanofenfos and azinphos-ethyl. Except for tolcofos-methyl which is a fungicide, the rest are insecticides. These pesticides are frequently used by vegetable farmers and commonly detected in residue surveys. The pesticides were fortified at 0.5 ppm level and the results are given in Table 1. The recoveries for ten OP pesticides were within the acceptable range of 70 - 120% (Parker 1991). The recovery for these pesticides ranged from 94.3 % - 108.7% with coefficient of variation (CV) of 3.1% to 8.1%. A low recovery of 17.3% was obtained for the polar pesticide, methamidophos. This is due to strong retention of this pesticide by the polar SPE anion exchange sorbent, SAX/NH_2. Methamidophos is a polar pesticide with solubility of 200,000 mg/L in water. Dimethoate is less polar with solubility of 25,000 mg/L while solubility for other OP pesticides tested is in the range of 0.05 mg/L to 40 mg/L.

TABLE 1
Recovery of organophosphorus pesticides

Pesticide	Recovery[(a)] %	CV %
Methamidophos	17.3	4.2
Dimethoate	108.7	5.9
Diazinon	104.3	5.5
Tolcofos-methyl	103.3	8.1
Fenitrothion	95.0	4.6
Chlorpyrifos	102.3	4.6
Phenthoate	105.7	4.9
Prothiofos	102.7	4.9
Triazofos	103.7	4.0
Cyanofenfos	101.7	3.2
Azinphos-ethyl	94.3	3.1

(a) : n = 3
CV : coefficient of variation

Method Validation

Three types of vegetables: carrot, cucumber and green mustard which represent root, cucurbits and brassica families respectively were selected for this study. Each group contains different chemical compounds which may co-elute with the pesticide. This may interfere with the GC analysis of sample extracts.

a. Carrot

Recoveries of OP pesticides from fortified carrot samples using SAX/NH_2 cleanup and without cleanup are shown in Table 2. For the method without cleanup, the recoveries for eleven pesticides were within the acceptable range. The recoveries for these pesticides were from 76% to 108.3% with CV of 5.0 - 11.0%. Comparable results were obtained for SPE SAX/NH_2 cleanup method for nine OP pesticides. The recoveries ranged from 92.0% to 115.0% with CV of 4.0 - 7.2%. Low recoveries were observed for methamidophos and dimethoate. This is due to strong retention by polar anion exchange sorbents, SAX/NH_2 as these pesticides are more polar compared to the other OP pesticides. Statistical analysis showed that the t-value for nine pesticides was 7.13 which was higher than the critical value. Therefore, there was a significant difference between the SPE method and the current method without cleanup. The SPE cleanup method results in improvement in the recoveries.

The chromatograms for carrot samples using SAX/NH_2 cleanup and without cleanup are shown in *Figs. 1* and *2*. Both chromatograms showed no interference peaks co-eluted with OP pesticides because FPD in phosphorus mode is selective and specific responding to phosphorus compounds. The colour of the extract without cleanup was red while after the cleanup it was yellow. The glass wool in the liner and the head of the GC column were cleaner after several injections compared to the method without cleanup. This effect is more prominent and is reflected in the chromatogram when a less specific detector

TABLE 2
Recovery of organophosphorus pesticides from spiked carrot samples with and without SAX/NH_2 cleanup

Pesticide	SAX/NH_2		No cleanup	
	Recovery[(a)] %	CV %	Recovery[(a)] %	CV %
Methamidophos*	0	0	76.0	5.0
Dimethoate*	30.3	17.0	108.3	6.0
Diazinon	92.0	6.1	89.3	9.3
Tolcofos-methyl	113.3	5.8	98.7	10.7
Fenitrothion	108.7	5.7	102.0	6.1
Chlorpyrifos	112.3	5.8	99.3	11.0
Phenthoate	109.7	5.7	97.0	8.9
Prothiofos	115.0	7.2	103.7	10.0
Triazofos	105.3	4.0	95.7	8.1
Cyanofenfos	112.7	4.0	99.7	7.2
Azinphos-ethyl	102.3	6.8	97.3	9.2
AV	107.9		98.1	
SD	7.2		4.1	

AV — Average mean excluding *
SD — Standard deviation excluding *
CV — Coefficient of variation
(a) — n = 3

such as electron capture detector is used. The advantage of the SPE method over the current method without cleanup is that the former removed a substantial amount of co-extractives from the samples. This can reduce the cost of GC maintenance especially for the GC inlet and column. Without the SPE cleanup, the glass wool insert in the liner needs to be replaced very often and the liner needs to be cleaned regularly. Contaminants significantly reduce the performance and lifetime of chromatography columns. The other advantage of SPE is that less solvent is used compared to conventional cleanup methods such as gel permeation chromatography (GPC) which requires large amounts of solvent and is time consuming. Also, cross-contamination can be eliminated as the SAX/NH_2 tube is discarded after use.

b. Cucumber

Recoveries of OP pesticides from fortified cucumber samples using SAX/NH_2 and without cleanup are given in Table 3. The recoveries for 9 OP pesticides using cleanup were within the acceptable range. They ranged from 80.3% to 95.3% with CV of 2.5% to 7.1%. A lower recovery was obtained for dimethoate while all the methamidophos was lost during the cleanup. This is due to strong retention of these pesticides by the polar anion exchange sorbent, SAX/NH_2 as they are more polar compared to other OP pesticides. As a result, they were not eluted by the polar solvent. The recoveries for the method without cleanup ranged from 69.3% to 111.0% with CV of 1.2% to 6.2%. Statistical analysis

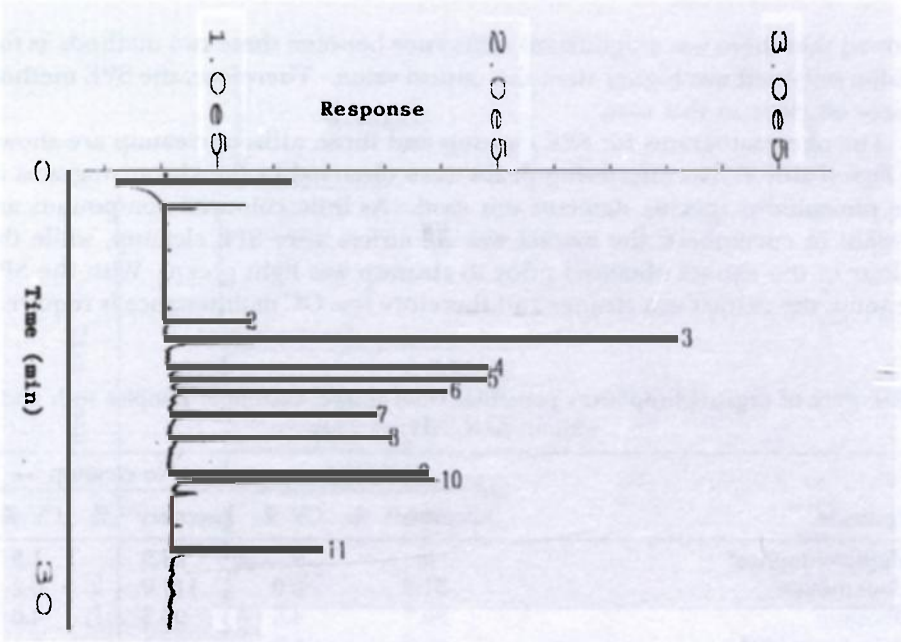

Fig. 1: GC chromatogram of carrot extract after SPE cleanup.
Peak: 1, methamidophos; 2, dimethoate; 3, diazinon; 4, tolcofos-methyl; 5, fenitrothion; 6, chlorpyrifos; 7, phenthoate; 8, prothiofos; 9, triazophos; 10, cyanofenfos, 11, azinphos-methyl

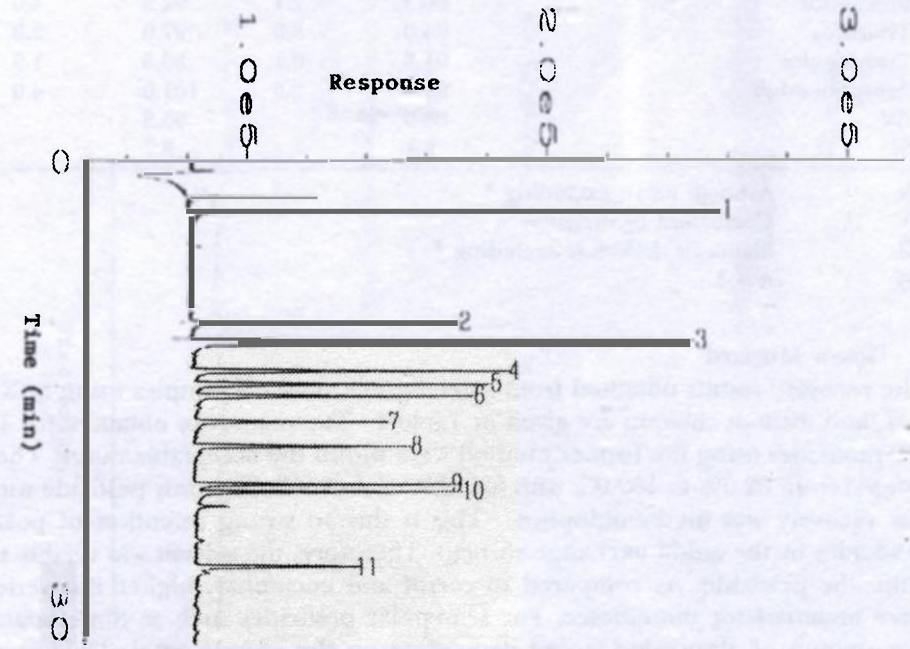

Fig. 2: GC chromatogram of carrot extract without cleanup.
Peak: 1, methamidophos; 2, dimethoate; 3, diazinon; 4, tolcofos-methyl; 5, fenitrothion; 6, chlorpyrifos; 7, phenthoate; 8, prothiofos; 9, triazophos; cyanofenfos, 11, azinphos-methyl

showed that there was a significant difference between these two methods as the t-value obtained was higher than the critical value. Therefore, the SPE method is less efficient in this case.

The chromatograms for SPE cleanup and those without cleanup are shown in *Figs. 3* and *4*. No interfering peaks were observed in the chromatograms as the phosphoros specific detector was used. As little coloured compounds are present in cucumbers, the extract was colourless after SPE cleanup, while the colour of the extract obtained prior to cleanup was light green. With the SPE cleanup, the extract was cleaner and therefore less GC maintenance is required.

TABLE 3
Recovery of organophosphorus pesticides from spiked cucumber samples with and without SAX/NH_2 cleanup

	SAX/NH_2		No cleanup	
Pesticide	Recovery[(a)] %	CV %	Recovery[(a)] %	CV %
Methamidophos*	0	0	69.3	1.5
Dimethoate*	51.7	6.0	111.0	6.2
Diazinon	86.7	4.6	96.3	4.0
Tolcofos-methyl	87.0	5.3	94.3	3.1
Fenitrothion	89.0	6.1	98.3	1.5
Chlorpyrifos	82.0	7.0	91.7	4.2
Phenthoate	95.3	5.9	97.3	1.2
Prothiofos	80.3	7.1	92.3	4.0
Triazofos	94.0	3.5	97.0	2.0
Cyanofenfos	91.3	6.1	89.3	1.5
Azinphos-ethyl	91.3	2.5	101.0	4.0
AV	88.5		95.3	
SD	5.1		3.7	

AV — Average mean excluding *
CV — Coefficient of variation
SD — Standard deviation excluding *
(a) — n = 3

c. Green Mustard

The recovery results obtained from spiked green mustard samples using SAX/NH_2 and without cleanup are given in Table 4. The recoveries obtained for 10 OP pesticides using the former method were within the acceptable range. They ranged from 82.0% to 102.0% with CV of 2.7% to 9.9%. The only pesticide with low recovery was methamidophos. This is due to strong retention of polar pesticides by the anion exchange sorbent. Therefore, the solvent was unable to elute the pesticide. As compared to carrot and cucumber, higher recoveries were obtained for dimethoate. For semi-polar pesticides such as dimethoate, the amount of absorption varied depending on the sample types. Therefore, there was wide variation among the three types of vegetables tested. The recoveries obtained for the method without cleanup were between 74.3% to

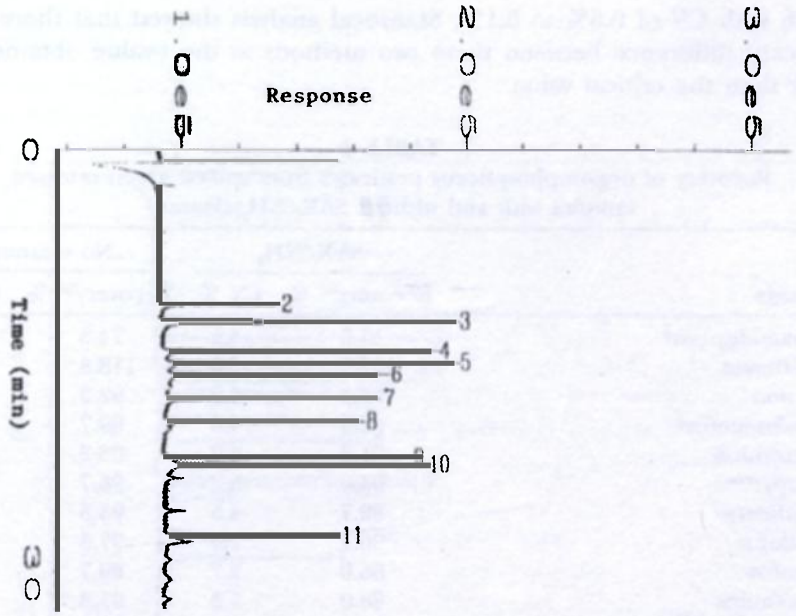

Fig. 3: GC chromatogram of cucumber extract after SPE cleanup.
Peak: 1, methamidophos; 2, dimethoate; 3, diazinon; 4, tolcofos-methyl; 5, fenitrothion; 6, chlorpyrifos; 7, phenthoate; 8, prothiofos; 9, triazophos; 10, cyanofenfos, 11, azinphos-methyl

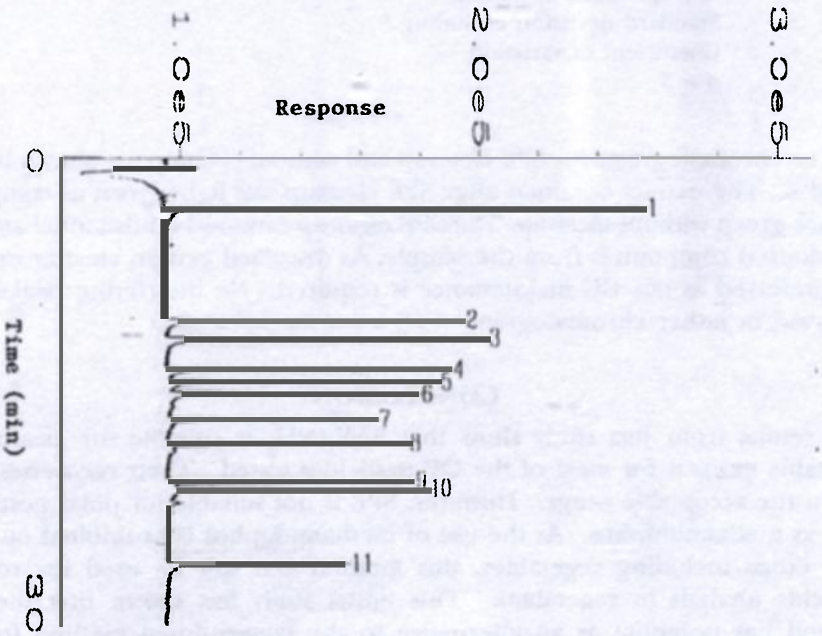

Fig. 4: GC chromatogram of cucumber extract without cleanup.
Peak: 1, methamidophos; 2, dimethoate; 3, diazinon; 4, tolcofos-methyl; 5, fenitrothion; 6, chlorpyrifos; 7, phenthoate; 8, prothiofos; 9, triazophos; 10, cyanofenfos, 11, azinphos-methyl

118.3% with CV of 0.6% to 6.1%. Statistical analysis showed that there was a significant difference between these two methods as the t-value obtained was higher than the critical value.

TABLE 4
Recovery of organophosphorus pesticides from spiked green mustard samples with and without SAX/NH_2 cleanup

Pesticide	SAX/NH_2		No cleanup	
	Recovery[(a)] %	CV %	Recovery[(a)] %	CV %
Methamidophos*	15.3	4.5	74.3	1.5
Dimethoate	102.0	9.9	118.3	6.1
Diazinon	88.3	6.5	92.3	1.5
Tolcofos-methyl	92.0	4.6	89.7	1.5
Fenitrothion	91.3	4.7	93.3	5.9
Chlorpyrifos	94.0	5.2	96.7	2.1
Phenthoate	89.7	4.5	93.3	3.2
Prothiofos	90.7	7.4	97.3	0.6
Triazofos	86.0	2.7	89.7	4.0
Cyanofenfos	88.0	5.3	91.3	3.2
Azinphos-ethyl	82.0	4.6	92.3	6.1
AV	90.4		95.5	
SD	5.3		8.4	

AV Average mean excluding *
SD Standard deviation excluding *
CV Coefficient of variation
(a) n = 3

The chromatograms for SPE cleanup and without cleanup are shown in *Figs. 5* and *6*. The extract obtained after SPE cleanup was light green as compared to dark green without cleanup. The SPE cleanup removed a substantial amount of coloured compounds from the sample. As discussed earlier, cleaner extracts are preferred as less GC maintenance is required. No interfering peaks were observed in either chromatogram.

CONCLUSION

The results from this study show that SAX/NH_2 is suitable for cleanup of vegetable extracts for most of the OP pesticides tested. Their recoveries were within the acceptable range. However, SPE is not suitable for polar pesticides such as methamidophos. As the use of methamidophos is prohibited on most food crops including vegetables, this method can still be used for routine pesticide analysis in vegetables. This initial study has shown that the SPE method has potential as an alternative to the conventional method for OP pesticide analysis in vegetable and fruit crops. The SPE method affords a cleaner extract and as such it reduces GC maintenance, especially on capillary columns and the injector inlet. This clean extract may also be suitable for ECD

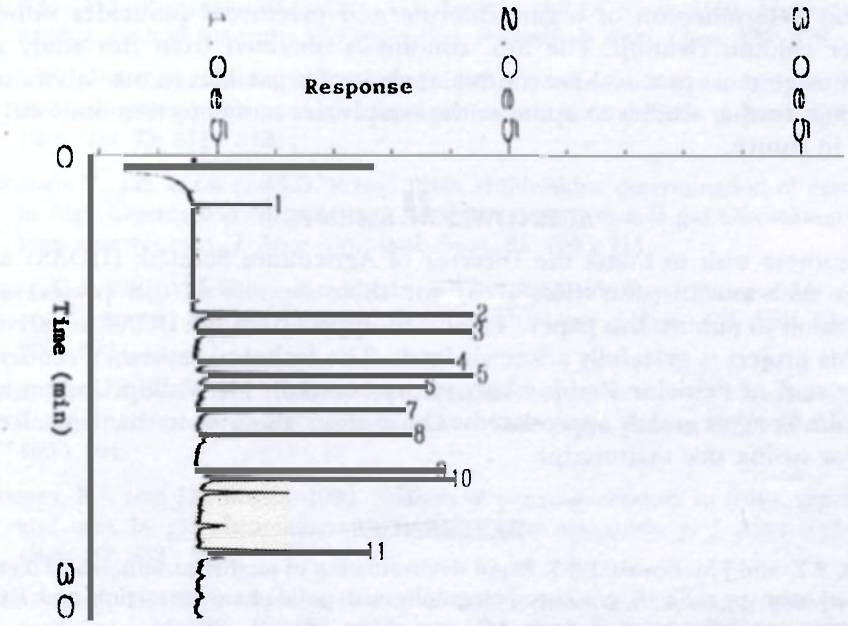

Fig. 5: GC chromatogram of green mustard extract after SPE cleanup.
Peak: 1, methamidophos; 2, dimethoate; 3, diazinon; 4, tolcofos-methyl; 5, fenitrothion; 6, chlorpyrifos; 7, phenthoate; 8, prothiofos; 9, triazophos; 10, cyanofenfos, 11, azinphos-methyl

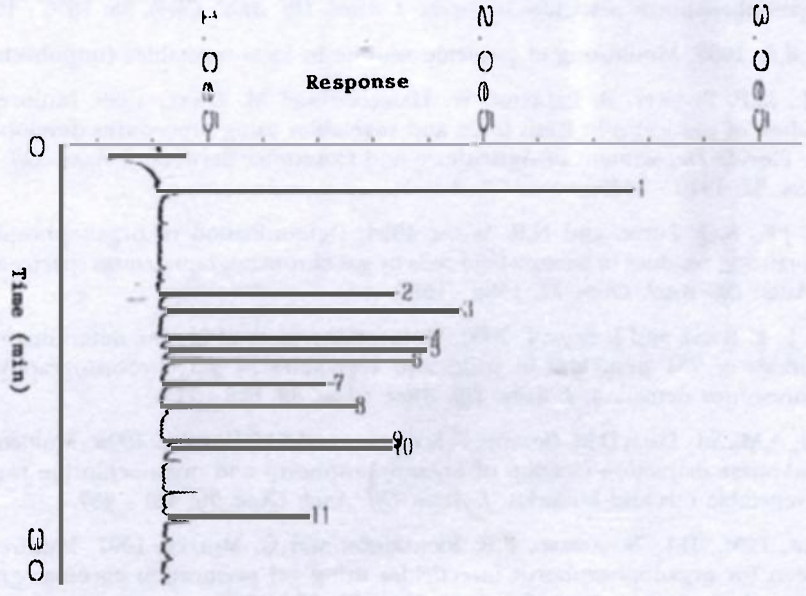

Fig. 6: GC chromatogram of green mustard extract without cleanup.
Peak: 1, methamidophos; 2, dimethoate; 3, diazinon; 4, tolcofos-methyl; 5, fenitrothion; 6, chlorpyrifos; 7, phenthoate; 8, prothiofos; 9, triazophos; 10, cyanofenfos, 11, azinphos-methyl

method determination of organochlorine and pyrethroid pesticides without further column cleanup. The SPE conditions obtained from this study are found to be most practical for routine analysis of vegetables in our laboratory. However, further studies to optimise the sample size and detection limit will be done in future.

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Determination of Pyrethroid Pesticides in Vegetables by Solid-Phase Extraction Cleanup and Gas Chromatography

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ABSTRAK

Satu kaedah yang ringkas dan cepat telah dihasilkan untuk menentukan lima jenis racun perosak pirethroid pada sayur-sayuran. Sisa baki racun perosak diekstrak dengan aseton dan metilena klorida. Ekstrak dibersihkan dengan turus ekstraksi fasa pepejal amina kuaterner dan aminopropil. Racun perosak ditentukan dengan kromatografi gas yang dilengkapkan dengan pengesan tangkapan elektron. Pemulihan daripada tiga jenis sayur-sayuran iaitu lobak merah, timun dan sawi hijau pada aras 0.25 - 0.5 mg/kg adalah di antara 72.0 dan 117.0%. Keputusan ini adalah setanding dengan yang diperoleh daripada kaedah pembersihan melalui gel silika.

ABSTRACT

A simple and rapid method has been developed for the simultaneous determination of five pyrethroid pesticides in vegetables. Residues were extracted with acetone and methylene chloride. Extracts were cleaned up by solid-phase extraction mixed-mode column using quaternary amine and aminopropyl sorbents. The pesticides were determined by gas chromatography with electron capture detector. The recoveries from three vegetables: carrot, cucumber and green mustard, spiked at 0.25-0.5 mg/kg were 72.0 to 117.0%. The results were comparable to those obtained by the silica gel cleanup method.

Keywords: Solid-phase extraction, pyrethroid pesticides, gas chromatography

INTRODUCTION

The use of pyrethroid insecticides in agriculture has increased rapidly since the development of the first pyrethroid, permethrin, in the 1970s. This is because of their desirable environmental properties of short persistence and non-toxicity to mammals. These features, combined with their broad spectrum of insecticidal activity and the comparatively low application rates required for insect control, have made the pyrethroid an environmentally safe and acceptable alternative to organochlorine pesticides. In Malaysia, they are widely used for vegetable cultivation throughout the growing season. As these insecticides have a shorter preharvest interval of between 3 - 5 days, they are the preferred choice of insecticides near harvest, as they do not pose a residue problem. Based on the residue survey carried out by the Department of Agriculture, Sarawak from 1987 - 2000, these insecticides constitute only a small portion of the total violation even though they are the most widely used pesticides by vegetable farmers in Sarawak (Chai 2000).

Pyrethroid pesticides are usually more difficult to determine than the organophosphates because of their appreciably lower levels of application. These compounds consist of a mixture of stereoisomers. Therefore, separation is more easily carried out on capillary column. Several methods for the determination of pyrethroids have been reported. Baker *et al.* (1982) developed a multi-residue method for the determination of pyrethroids in fruit and vegetables. Residues were first extracted with hexane and acetone. The extracts were then cleaned up on silica gel column and determined by gas chromatography (GC) with electron capture detector (ECD) and liquid chromatography (LC). Procedures based on GC have also been reported by Bolgyo *et al.* (1983) for the determination of six pyrethroid pesticides in fruits, vegetables and leaves by using acetone and methylene chloride extraction and carbon-magnesia-diatomaceous earth and alumina cleanup. Bottomley *et al.* (1984) have determined pyrethroid pesticides in grain using LC. Samples were extracted with acetone and methanol and cleaned up on aluminium oxide column.

Pang *et al.* (1994) described a simple and rapid packed column GC method for the simultaneous determination of ten pyrethroid insecticides in grains, fruits and vegetables using acetone and petroleum ether extraction. The extracts were cleaned up on a florisil column or a column consisting of a mixture of florisil, charcoal and alumina. A GC method for the determination of nine pyrethroid insecticides in products of animal origin was reported (Pang *et al.* 1994). Pang *et al.* (1995) determined nine pyrethroid insecticides in fruits and vegetables using methanol and toluene as extractants followed by florisil and charcoal column chromatography cleanup. Braun *et al.* (1982) reported a multi-residue method for the determination of pyrethroid pesticides in celery and animal products.

Numerous methods for the determination of the residues of pyrethroid pesticides in different matrices using solid phase extraction (SPE) cleanup have been developed. Bennett *et al.* (1997) developed a method for the determination of permethrin and other pesticides in liquid whole milk using octadecyl (C18) and aminopropyl (NH_2) silica cartridges. Jasson *et al.* (2000) reported good recoveries for twenty six pesticides including pyrethroid pesticides, lamda-cyhalothrin, cyfluthrin, esfenvalerate and deltamethrin in honey using Isolute ENV+. Mogadati *et al.* (1999) described a method using C18 and LC-amino for the cleanup of soil extracts prior to gas chromatography/mass spectrometry (GC/MS) determination. Sheridan *et al.* (1999) applied GC/MS for the determination of pyrethroid pesticides in fruits, vegetables and milk using quaternary amine (SAX) and ethylenediamine-N-propyl (PSA) as sorbents. Cook *et al.* (1999) reported C18, florisil and aminopropyl SPE cleanup for the determination of cypermethrin, fenvalerate, permethrin and other pesticides in fruits and vegetables. Fillion *et al.* (2000) described the removal of co-extractives in fruits and vegetables using C18 and NH_2 SPE cleanup, followed by GC/MS and LC fluorescence detection.

This paper reports the extraction of pyrethroid pesticides in vegetables using acetone and methylene chloride followed by clean up on SPE SAX/NH_2 and determination on GC-ECD. The results obtained were compared to the current method using silica gel column chromatography cleanup.

METHOD

Chemicals and Reagents

All the chemicals and reagents were of analytical grade. Pesticide standards were obtained from Dr. Ehrenstorfer™, Germany. SPE tubes, Isolute SAX/NH_2 (1 g sorbent in 6 mL tube) were purchased from International Sorbent Technology.

Instrumentation

A Hewlett-Packard 6890 gas chromatograph equipped with electron capture detector was used for the determination of pyrethroid pesticides. GC conditions were : injector temperature, 260°C; detector, 280°C; carrier flow (nitrogen) 1.2 mL/min; oven temperature, 120°C (0.5 min), rate 10°C/min to 180°C, rate 6°C/min to 240°C, rate 10°C/min to 280°C (12 min). Pyrethroid pesticides were analysed on HP Ultra 1, 25 m x 0.32 mm x 0.5 mm column.

Procedures

The method was validated using five pyrethroid pesticides, namely lamda-cyhalothrin, permethrin, cypermethrin, fenvelerate and deltamethrin fortified in carrot (*Daucus carota*), cucumber (*Cucumis sativus*) and green mustard (*Brassica chinensis*). Each sample was fortified with 0.5 ppm of each pesticide except lamda-cyhalothrin with 0.25 ppm. Three replicate fortifications for each matrix type were prepared.

Extraction was carried out using procedures described by Steinwandter (1985). A weight of 50 g sample was homogenised in a blender containing 100 mL acetone, 75 mL dichloromethane and 15 g sodium chloride for 3 min. The homogenised mixture was allowed to separate into organic and aqueous layers. The organic layer was transferred to a beaker and 3 g of sodium sulphate was added to remove any remaining water.

For the current method, 2 mL of extract was transferred to a chromatographic column filled with 10 g of silica gel. The column was eluted with 80 mL of methylene chloride-hexane mixture (1:1 v/v). The eluant was analysed for pyrethroid pesticides on GC-ECD.

For the SPE method, SAX/NH_2 sorbent was used for pyrethroid cleanup. The SAX/NH_2 was conditioned with 10 mL of acetone : petroleum ether (1:2). 2 mL of the extract was transferred to the SPE tube. The SPE tube was eluted with 10 mL of conditioning solvent at a flow rate of 1 mL/min. The eluant was analysed for pyrethroid pesticides using GC-ECD.

Statistical Analysis

The t-test was used to determine the significance of the difference between the current method and SPE method in terms of recoveries. They were considered significantly different when values of t (calculated) > t (critical) at 95% confidence level.

RESULTS AND DISCUSSION

Optimisation of SPE Conditions

The SPE conditions were optimised to obtain good recoveries for pyrethroid pesticides. Among the parameters studied were flow rate and solvent polarity. The studies concluded that a flow rate of 1 mL/min to 1.5 mL/min was sufficient to recover the pyrethroid pesticides above 67% when the combination of acetone/hexane or acetone/petroleum ether was used. The flow rate can be increased to 3 - 4 mL/min without affecting the efficiency of recovery. Flow rates of less than 1 mL/min resulted in low recoveries of less than 35% for all the pesticides studied.

Development of an optimum solvent system for pyrethroid pesticides was based on several criteria. Solvents, which were hazardous or expensive to be disposed of, were not evaluated. A volatile solvent system must be used, as rapid evaporation of a large volume of solvent would be required in sample preparation without causing the loss of volatile pesticides. The solvent system must be sufficiently polar to extract most polar pesticides. The final sample extract should have only minimal co-extractives interference.

Solvent mixtures consisting of any two of the following solvents, namely acetone, hexane and petroleum ether in various ratios (v/v) were used in this study. Low polarity solvent mixture such as acetone : hexane (5:95) resulted in excellent recoveries for pyrethroid pesticides. Increasing the solvents' polarity did not result in losses of pesticides. An attempt was made to use more polar eluting solvents such as acetone : petroleum ether (4:1). This resulted in good recoveries for all the pyrethroid pesticides studied. The critical factor to be observed was that the SPE tube should not be left dry after the samples were eluted. A volume of 10 mL of eluting solvents was sufficient to condition the SPE tube and to elute the analytes. Increase of the volume of solvent for elution has not improved the recoveries.

The study has found the optimal conditions for the extraction of pyrethroid pesticides to be as follows : (a) solvent mixture : acetone : petroleum ether (1:2); (b) volume of eluting solvent : 10 mL; (c) flow rate of elution : 1 mL/min. The study was conducted at room temperature (25°C).

Recovery Studies

Five pyrethroid pesticides, namely lamda-cyhalothrin, permethrin, cypermethrin, fenvelerate and deltamethrin, were selected for this study. A concentration of 0.5 ppm of pesticides was leached through SPE, SAX/NH_2 using the optimum conditions. The results are given in Table 1. The recoveries for pyrethroid

pesticides ranged from 67.3 to 114.7% with the coefficient of variation (CV) ranging from 3.1 to 11.5%.

Method Validation

Three types of vegetables, namely carrot, cucumber and green mustard, which represent root, cucurbits and brassica family, were selected for this study. As each group contains different chemical compounds, they may co-elute with the pyrethroid pesticides. This may interfere with the GC analysis of sample extracts.

a. Carrot

Recoveries of pyrethroid pesticides from carrot samples using SAX/NH_2 and silica gel column chromatography cleanup are shown in Table 2. For silica gel cleanup (the reference method), the recoveries ranged from 91.0 to 116.7% with CV of 1.2 to 6.7%. Comparable results were obtained from SPE SAX/NH_2 cleanup method with recoveries ranging from 92.3 to 117% with CV of 3.5 to 11.4%. When comparing these two methods, the t-value obtained was 0.65 with 4 degrees of freedom. As this value was lower than the calculated t-value, there was no significant difference between these two methods.

Chromatograms for carrot samples using SAX/NH_2 and silica gel cleanup are shown in *Figs. 1* and *2*, respectively. Both chromatograms showed no interference to peaks identified for pyrethroid pesticides. However, more unknown peaks were observed in the GC chromatogram of carrot extract after SPE cleanup than that after silica gel cleanup. This may be due to smaller amounts of sorbent used in SPE cleanup (1 g) than those in silica gel cleanup (10 g). The colour of both extracts was yellow. It is proposed that additional cleanup using SPE solvent such as C18 is required to remove the excessive co-extractives present in the sample. Several researchers have used more than two sorbents for the removal of co-extractives (Sheridan and Meola 1999; Cook *et al.* 1999; Fillion *et al.* 2000). Alternatively, a smaller sample size of 10 - 20 g is suggested instead of 50 g. This can reduce the matrix interferences substantially. In the SPE cleanup method, only 10 mL of solvent was used as compared to

TABLE 1
Recovery of pyrethroid pesticides

Pesticide	Recovery[(a)] %	CV %
Lamda-cyhalothrin	85.3	9.5
Permethrin	103.0	11.5
Cypermethrin	114.7	3.1
Fenvalerate	110.3	4.9
Deltamethrin	67.3	3.2

(a) : n = 3
CV : coefficient of variation

80 mL in the conventional silica gel cleanup. The SPE cleanup allows automation and thus shortens the time needed for the analysis. These advantages in the SPE method will be most welcome in routine analysis that requires high output. Contamination can be eliminated in SPE as the tube is discarded after use. No hazardous solvent such as methylene chloride was used in the SPE method.

b. Cucumber

The results of pyrethroid pesticides recovery from spiked cucumber samples using the SAX/NH_2 and silica gel cleanup methods are given in Table 3. For the SAX/NH_2 method, the recoveries were 85 to 97.7% with CV of 3.5 to 9.0%.

TABLE 2
Recovery of pyrethroid pesticides from spiked carrot samples using SAX/NH_2 cleanup and silica gel cleanup

Pesticide	SAX/NH_2		Silica gel (reference method)	
	Recovery[a] %	CV %	Recovery[a] %	CV %
Lamda-cyhalothrin	105.7	11.4	100.3	1.2
Permethrin	96.0	3.6	106.3	2.1
Cypermethrin	106.0	3.5	111.7	6.7
Fenvalerate	117.0	3.5	116.7	5.7
Deltamethrin	92.3	4.2	91.0	3.6
AV	103.4		105.2	
SD	9.7		10.0	

AV : average mean
SD : standard deviation
CV : coefficient of variation
(a) : n = 3

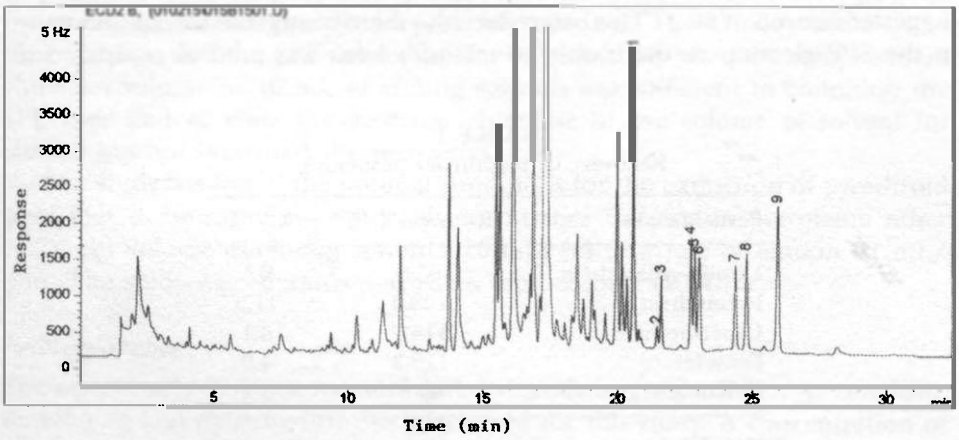

Fig. 1: GC chromatogram of carrot extract after SPE cleanup. Peaks: 1, lamda-cyhalothrin; 2, 3, permethrin; 4, 5, 6, cypermethrin; 7, 8, fenvalerate; 9, deltamethrin

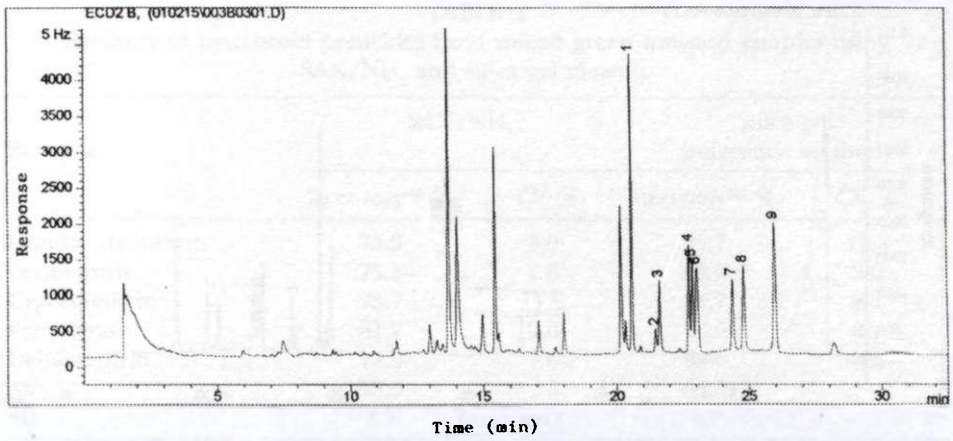

Fig. 2: GC chromatogram of carrot extract after silica gel cleanup. Peaks: 1, lamda-cyhalothrin; 2, 3, permethrin; 4, 5, 6, cypermethrin; 7, 8, fenvalerate; 9, deltamethrin

Corresponding results of 76.3 to 100.0% were obtained for the silica gel method. The CV was between 2.7 to 7.6%. Statistical analysis showed that there was no significant difference between these two methods as the t-value obtained was lower than the critical value. The chromatograms for the SPE and silica gel cleanup methods are shown in *Figs. 3* and *4*, respectively. The extract of cucumber was colorless after the SPE or silica gel cleanup. The chromatograms showed no interfering peaks after cleanup. This is because cucumber contains less interfering compounds, which may respond more to ECD, compared to the carrot samples. It was concluded that single SPE SAX/NH_2 was sufficient for the cleanup of cucumber samples.

TABLE 3
Recovery of pyrethroid pesticides from spiked cucumber samples using SAX/NH_2 and silica gel cleanup

Pesticide	SAX/NH_2		Silica gel (reference method)	
	Recovery[a] %	CV %	Recovery[a] %	CV %
Lamda-cyhalothrin	85.0	3.5	76.3	3.5
Permethrin	93.0	3.5	85.0	2.7
Cypermethrin	93.0	5.6	91.7	5.0
Fenvalerate	97.7	9.0	100.0	7.0
Deltamethrin	96.3	8.7	90.0	7.6
AV	93.0		88.0	
SD	4.9		8.7	

AV : average mean
SD : standard deviation
CV : coefficient of variation
(a) : n = 3

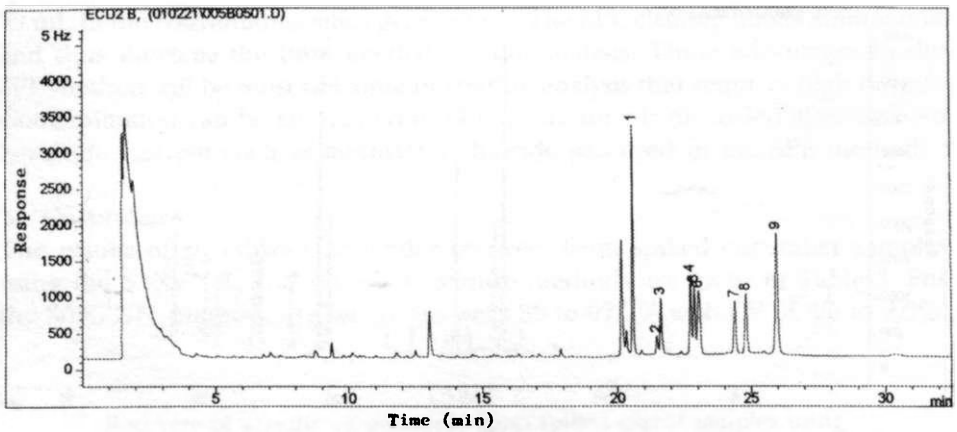

Fig. 3: GC chromatogram of cucumber extract after SPE cleanup. Peaks: 1, lamda-cyhalothrin; 2, 3, permethrin; 4, 5, 6, cypermethrin; 7, 8, fenvalerate; 9, deltamethrin

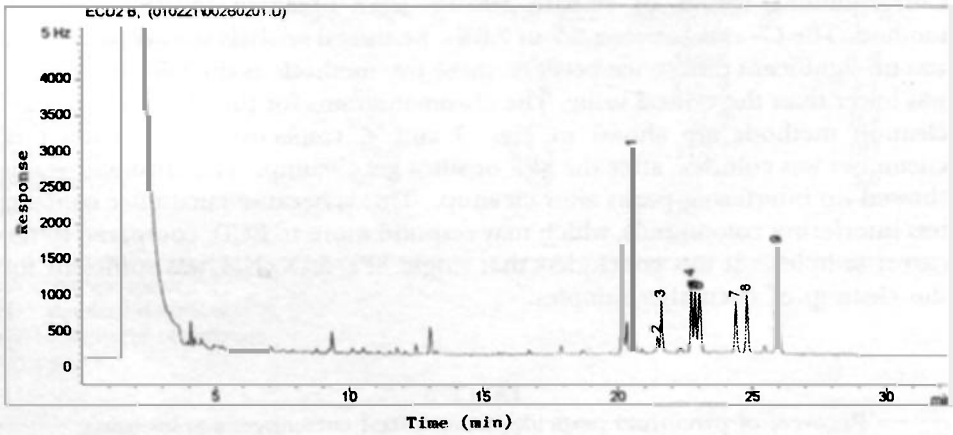

Fig. 4: GC chromatogram of cucumber extract after silica gel cleanup. Peaks: 1, lamda-cyhalothrin; 2, 3, permethrin; 4, 5, 6, cypermethrin; 7, 8, fenvalerate; 9, deltamethrin

c. Green Mustard

The results of pesticide recovery from spiked green mustard samples using SAX/NH_2 and silica gel cleanup are given in Table 4. The recoveries obtained from the SPE cleanup were slightly lower than those obtained from the silica gel cleanup. However, the recoveries were still within the acceptable range. The recoveries for the SAX/NH_2 cleanup were 72 to 81.7% with CV of 4.6 to 11.9%. Higher recoveries of 92 to 96% were obtained for the silica gel cleanup with CV of 3.0 to 11.2%. Statistical analysis showed that there was a significant difference between these two methods. Modification in terms of solvent mixture may improve the recoveries. Further optimisation work is required for these types of samples.

TABLE 4
Recovery of pyrethroid pesticides from spiked green mustard samples using SAX/NH_2 and silica gel cleanup

Pesticide	SAX/NH_2		Silica gel (reference method)	
	Recovery[(a)] %	CV %	Recovery[(a)] %	CV %
Lamda-cyhalothrin	75.0	6.0	95.7	11.2
Permethrin	75.3	4.6	92.0	3.0
Cypermethrin	73.7	11.9	94.7	8.7
Fenvalerate	81.7	9.6	96.0	8.7
Deltamethrin	72.0	7.6	93.0	6.2
AV	75.5		94.3	
SD	3.7		1.7	

AV : average mean
SD : standard deviation
CV : coefficient of variation
(a) : n = 3

The chromatograms for the SPE and silica gel cleanup are shown in *Figs. 5* and *6* respectively. The extract obtained after the SPE cleanup was light green as compared to yellow in the silica gel cleanup. Despite this, no interfering peak was observed in the SPE cleanup method and the chromatogram was comparable to that obtained from the silica gel cleanup. Apparently, both methods had successfully removed the interfering co-extractives prior to GC determination. It was concluded that only one single SPE sorbent was sufficient to perform the sample cleanup. Nonetheless, the relatively lower percentage of recovery from the SPE cleanup method needs further investigation.

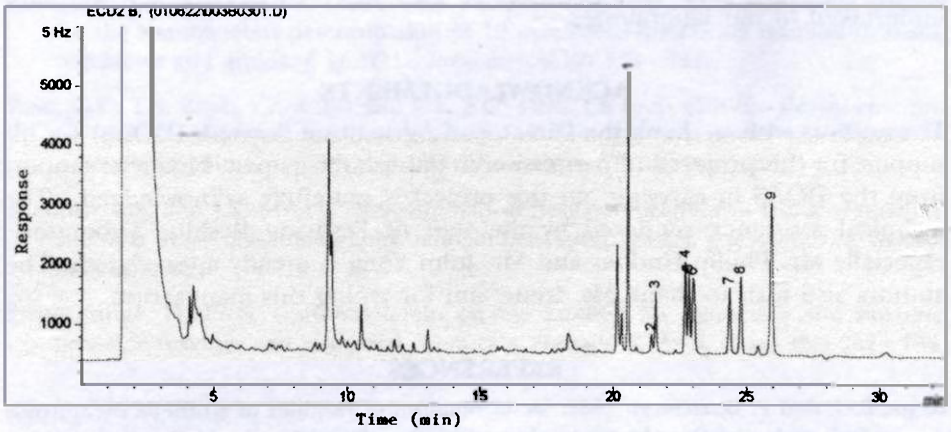

Fig. 5: GC chromatogram of green mustard extract after SPE cleanup. Peaks: 1, lamda-cyhalothrin; 2, 3, permethrin; 4, 5, 6, cypermethrin; 7, 8, fenvalerate; 9, deltamethrin

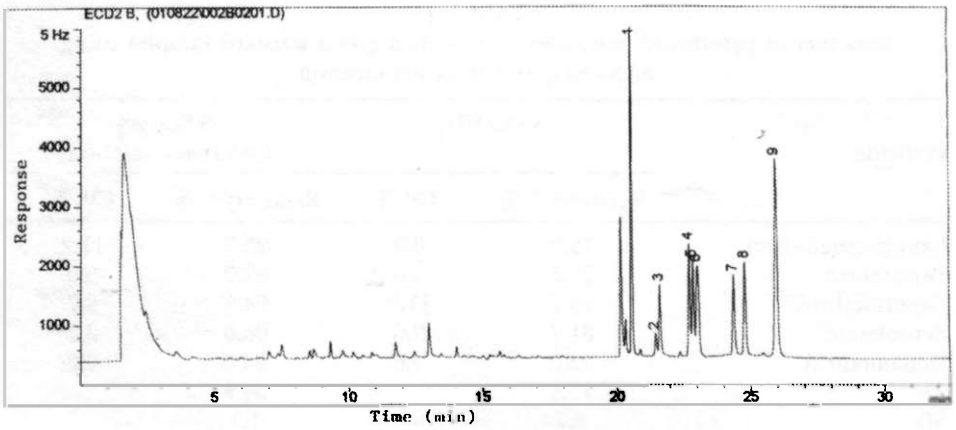

Fig. 6: GC chromatogram of green mustard extract after silica gel cleanup. Peaks: 1, lamda-cyhalothrin; 2, 3, permethrin; 4, 5, 6, cypermethrin; 7, 8, fenvalerate; 9, deltamethrin

CONCLUSION

The results from this study showed that SAX/NH_2 is suitable for the cleanup of vegetable extracts for pyrethroid pesticides. Recoveries obtained were comparable to those obtained from the established silica gel cleanup method. The SPE sorbent was found to have better efficiency than silica gel, particularly on the cleanup of cucumber and green mustard extracts. This study has shown that the SPE method has great potential as an alternative to the conventional silica gel cleanup method and it is possible to extend the application for the analysis of pesticides in a wider range of vegetables and fruit crops. The benefits of the SPE method are : less solvent consumption, no cross-contamination, shorter analysis time and the process can be automated. Further studies to optimise the sample size and detection limit are currently being undertaken in our laboratories.

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An Active Server Pages (ASP) Approach in Ecotourism Rating Expert System

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ABSTRAK

Alam semula jadi merupakan asas pengeluaran sektor pelancongan. Keuntungan industri pelancongan bergantung kepada daya tarikan destinasi pelancongan. Malaysia adalah sebuah negara yang dikurniakan dengan keajaiban tropikal yang kaya dengan pelbagai tarikan ekologikal dan tentunya berpotensi untuk pelancongan. Justeru itu, status alam semula jadi memainkan peranan yang kritikal untuk mengekalkan industri ini. Konsep eko-pelancongan mengutamakan kepentingan ekologi pada tahap maksimum. Aspek ini walaupun mempunyai potensi yang khas untuk pembangunan dan eksploitasi, ianya masih belum menerima perhatian yang sepenuhnya di Malaysia. Semua eko-pelancongan harus dirancang, dipimpin dan diterokai dengan penuh pengawasan dan pengawalan untuk memastikan pengurusan yang berkesan dan cekap. Satu sistem pakar telah dihasilkan untuk menilai dan mengenal pasti tahap-tahap tertentu berlaraskan kepada beberapa kategori untuk megenal pasti kekalan destinasi eko-pelancongan di Semenajung Malaysia. Sistem pakar yang dibina berasaskan "*web-based information sharing platform*", berpandukan undang-undang keselamatan, kesihatan dan alam semula jadi yang sedia ada. Sistem ini direka dan dibina menggunakan "*back-end on-line database*" yang akan merekodkan semua urusan yang berjaya. Kertas kerja ini membentangkan sebuah model yang menggunakan cara skriptasi "*Active Server Pages*" untuk menguruskan dan mengeluarkan sistem penilaian pakar eko-pelancongan.

ABSTRACT

The environment is the backbone of tourism products. Profitability in tourism depends on maintaining the attractiveness of the tourist destinations. Malaysia is a tropical wonderland that is substantially rich in a variety of ecological pursuits and certainly of much tourist potential. Hence, the status of the environment plays a critical role if the industry is to sustain itself. The ecotourism concept emphasises the maximisation of the ecological interest areas. This aspect, while having vast potential for further development and exploitation, has not received adequate attention in Malaysia. All ecotourism sites must be planned, guided and exploited in a monitored and controlled manner for effective and efficient management. A systematic expert rating system is developed to maintain a certain level of standards based on different levels of categorisation to ensure the sustainability of ecotourism sites in Peninsular Malaysia. The expert system developed uses a web-based information-

sharing platform in line with the existing legislation on safety, health and environment. The system is designed and developed on a back-end on-line database, which will keep record of all successful transactions. This paper presents an efficient model using Active Server Pages scripting method to manage and deliver the ecotourism rating expert system.

Keywords: Expert system, web-based, knowledge-based, active server pages, ecotourism, sustainable management, rating

INTRODUCTION

As the tourism industry evolved, a new type of tourism, "ecotourism", has emerged and is making its mark in Malaysia. According to Cater and Lowman (1994), ecotourism evolved from nature tourism, which can be defined as ecologically sustainable tourism with the primary focus on experiencing natural areas. Using the same definition, ecotourism can be defined as ecologically sustainable tourism with the primary focus on experiencing natural areas that fosters environmental and cultural understanding application and conservation. Ceballos-Lascurain (1993) defined ecotourism as environmentally responsible travel and visitation to relatively undisturbed natural areas, in order to enjoy and appreciate nature (and any accompanying cultural features - both past and present) that promotes conservation, has low negative visitor impact, and provides for beneficially active socio-economic involvement of local populations.

The ecotourism concept emphasises the maximisation of the ecological interest areas, which include marine parks and islands, national parks, recreational forest reserves, and other forests, mangrove sites, limestone hills and cave sites, rivers, waterfalls and lakes, beach sites and montane areas (Ceballos-Lascurain 1993). This aspect, while having vast potential for further development and exploitation, has not received adequate attention in Malaysia. All ecotourism sites must be planned, guided and exploited in a monitored and controlled manner. Effective and efficient management is necessary to ensure that the project is feasible on a sustainable basis.

The main problems in the current practice in ensuring sustainable development of the ecotourism industry are: (a) lack of effective and efficient sustainable management practice of the ecotourism sites, (b) lack of enforcement in ensuring the ecosystem is sustained, (c) insufficient environmental cum ecological expertise that incorporates the fundamentals of safety and health, and (d) lack of consistent approaches in implementing a mitigation abatement measure and in satisfying the requirement of national environmental regulatory authorities.

Hence, a reliable and consistent rating system is required to ensure the sustainability of these ecotourism sites. The rating system can be used to assist in intelligent decision-making in terms of identifying potential ecotourism sites, which are not adhering to the standards of safety, health and environment. The systematic rating system can maintain a certain level of standards based on different levels of categorisation.

In order to make ecotourism development sustainable and to keep its impact at a desired level for future development, it is important that the present trend of the use of visiting areas is evaluated and rated. At the same time, resource assessment in terms of importance and attractiveness has also to be implemented. This study is the first in Malaysia in attempting to rate the ecotourism industry based on safety, health and environment towards resources and tourism development using a Web-based expert system developed by the researchers.

Therefore, the objectives of this research are: (a) to design, develop, test and implement an expert system to rate the ecotourism components consistently and reliably based on safety, health and environment, and (b) to extract the opinions of the domain experts, existing statistics and literature and field survey of people's perception in order to produce a set of rules to enable the expert system to be used to make intelligent decisions.

This paper presents an efficient model using Microsoft Access 2000 database software and an Active Server Pages (ASP) scripting method to manage and deliver the ecotourism rating system. Providing dynamic data on the Web, especially from a relational database, can be a daunting task because HTML is a mark-up language that has little processing power. Using ASP technology, it is now possible to provide dynamic Web access to local databases.

RATING SYSTEMS IN THE TOURISM INDUSTRY

Evaluation of the destination is essential in determining the best rating system. According to Pearce (1997), the multi-faceted nature of tourism and the complex nature of tourist demand make evaluation of the sector a complex task. Many different factors may influence where tourist development occurs. As tourist resources do not occur evenly or randomly in space, developers and planners will be faced with such practical questions as assessing the feasibility of developing a particular site. The result from the evaluation of the resources can be further developed to design effective and realistic rating system for the hospitality industry, in particular the tourism sector (Ritchie and Goeldner 1994).

Pearce further added that factors influencing the location of tourist projects or the tourist potential of an area could be grouped into the following categories, namely climate, physical conditions, attractions, access, existing facilities, land tenure and use. These factors are all interrelated.

In the tourism sector, a number of rating systems are used for accreditation of the site in terms of compliance of the local legislations. In 1995, the Alaskan Wilderness Recreation and Tourism Association, USA, emphasized the following 8 guidelines for the rating of the tourism sites:

1. Environmentally sustainable economic growth by minimising visitor impact on the wildlife, wild-land, native cultures and local communities.
2. Travel modes and facilities maintain low impact on the environment.
3. Businesses benefit the local economy and local inhabitants directly.

4. Business operations' effect on the environment is minimised.
5. All employees are educated on environmental impact.
6. Educational emphasis for tourists to learn.
7. Formula for the business and the guests to contribute to local non-profit effort for environmental protection.
8. Travel in the spirit of appreciation, participation and sensitivity.

In 1999, the Chinese National Tourism Administration (CNTA) classified and graded the tourist attractions according to hygiene, communications, safety and environment (Huang and Xiao 2000). The internationally recognised Blue-Flag criteria is another rating system widely used for beaches. The Blue-Flag criteria cover 4 major aspects of beach management, namely water quality, environmental education and information, environmental management and also safety and services (Font and Buckley 2001).

Another rating system was proposed by Shores (1999) for ecotourism scaling. He established a 0-5 scale to classify the stages of ecotourism. The proposed non-cumulative scaling measured different attributes and different levels.

One of the pioneer and most successful nations in managing ecotourism is Costa Rica. According to Font and Buckley (2001), the Costa Rican Tourism Institute, which is regulated by the Costa Rican National Accreditation Commission, introduced the Certification in Sustainable Tourism (CST) in 1999. The 5-level scaling is done for tourism-based companies and hotels. For the tourism companies, the 4 criteria evaluated are physical-biological parameters, infrastructure and services, external clients and the socio-economic environment. For the hotels, evaluation is done for physical-biological parameters, hotel facilities, customers and the socio-economic environment.

In 1994, the Tourism Authority of Thailand developed a rating and ranking system for the 109-tourism destinations using 5 criteria (SIRG 1997). The criteria used are:

1. resource attraction
2. susceptibility of impacts
3. opportunity for developing interpretation and educational programmes
4. diversity of ecotourist activity
5. compatibility of ecotourism with other tourism activities

The Malaysian National Ecotourism Plan, prepared by the World Wildlife Fund compiled a diagnostic list of ecotourism sites (WWF 1999). Table 1 outlines the diagnostic list.

WEB-BASED EXPERT SYSTEM TECHNOLOGY FOR RATING

It is easy to create Web pages to display static data from a database table. Most systems, such as Microsoft Access, include a "Save As HTML or Save As Web" feature to do just that. However, pages created in this way are a "snapshot in

time"; they do not change as the database is changed. We must re-create the pages each time changes are made to the data. Publishing and maintaining a large number of static Web pages is a maintenance nightmare (Lam 1997). In addition, users cannot search the database or choose particular items of data. Users can only see what was previously saved in HTML format.

Dynamic Web pages allow the user to connect to up-to-the-minute data, search the Web and display the data in different ways (Hatfield 1999). According to Yerkey (2000), four common ways of managing dynamic Web pages are, namely, Client-side Processing, Server-side Processing, Common Gateway Interface (CGI) and Active Server Pages (ASP).

TABLE 1
Diagnostic factors for ecotourism sites in the Malaysian Ecotourism Plan (WWF 1999)

	Diagnostic Factors
1.	Current tourism status
2.	Accessibility
3.	Existing facilities
4.	Flagship potential
5.	Development led by
6.	Type of attractions
7.	Type of activities
8.	Staff manning the site
9.	Rehabilitation and recovery needs
10.	Gross carrying capacity
11.	Current management agency

A simpler method of server-side processing is to use Active Server Pages (ASP). ASP is Microsoft's method of providing server-side processing for use by Web browsers. ASP embeds scripting statements directly onto the Web page, rather than by using a separate program. Being able to place ASP and HTML side by side can make coding much more manageable. As a result, ASP server-side scripting is browser-independent; a developer does not have to worry about browser dependencies, because only standard HTML will be delivered to the browser (Yerkey 2000).

The complexity of rating the components of the hospitality sector is evident with many factors to consider. With the aid of a Web accessed expert system, the rating procedure can be simplified. The World Wide Web has become the interface of choice for this information access in the 21st century.

The expert system developed uses a web-based information-sharing platform in line with the existing legislation on safety, health and environment. This diagnostic list from the Malaysian National Ecotourism Plan (WWF 1999) is used in enhancing the rating system.

EXPERT SYSTEM DEVELOPMENT METHODOLOGY

The initial study for this research project included a literature review on the existing rating system used in the hospitality and tourism sector. The findings of the research are combined with the opinions among domestic and foreign tourists and domain experts (including officers and ecotourism operators) to make the decision, and are to be converted into rules to be used as the basis for the construction of an expert system. Where no guidelines are available, recommendations based on the domain experts, literature and field survey statistics are incorporated. The expert system would use 3 levels of cumulative rating: the filtration module, basic compliance module and the advance compliance module. The domain experts would then be selected to determine the safety, health and environmental factors, which are critical in managing the ecotourism sites in Peninsular Malaysia. The knowledge derived from the expert opinions will be put into a series of analysis using the Delphi method (Birdir and Pearson 2000; Sahakian 1997) before deciding on the rules to be used by the expert system.

Data collection is carried out by the researcher and research assistants (RAs) from Universiti Putra Malaysia (UPM) and Taylor's College, School of Hospitality and Tourism (TCHT). The primary data required for the study is generated through the questionnaire survey of the domestic and foreign tourists to the study area. To strengthen the findings, the questionnaire survey is supplemented by reconnaissance survey and informal interviewing of the key informants.

In developing the questionnaire, previous studies on residents' perception and various other disciplines, namely, tourism and outdoor recreation, social and behavioural sciences, economics and management are obtained and used as guides. To measure the attitudes and perception, a five point Likert Scale (Murry and Hammons 1995) is used throughout the study. The decision to use the Likert Scale was based on the effectiveness of such a scale in previous studies of tourists' and residents' attitudes and perception (Wan Sabri 1987, Sengdeuane 1996).

All expert systems developed must fulfill 6 major development phases (Deborah *et al.* 1987; Durkin 1994). These iterative processes are incorporated in the rating system. They are Assessment (Phase 1), Knowledge Acquisition (Phase 2), Design (Phase 3), Testing (Phase 4), Documentation (Phase 5) and Maintenance (Phase 6). Similar development phase was also used by Giarrantano and Riley (1994) and Jackson (1992).

Phase 1: Assessment

A comprehensive literature review is done to determine the resource assessment in terms of the impact of the identified factors and the level of importance or weightage of these factors to ecotourism sites.

Phase 2: Knowledge Acquisition

The knowledge acquisition for the system is derived based on the domain expert consultation; secondary data of existing legislation and guidelines and

primary data based on field survey. Using the Delphi method (Birdir and Pearson 2000; Sahakian 1997), domain experts are consulted from:

a. Ministry of Culture, Arts and Tourism Malaysia
b. World Wildlife Fund for Nature (WWF) Malaysia
c. Forest Reserve Institute of Malaysia (FRIM) and Wildlife Department (PERHILITAN)
d. Taylor's College, School of Hospitality and Tourism and Sunway College, School of Hotel and Tourism
e. University of Toulouse, France

As for the secondary data, legislation and guidelines used to develop the rules for the inference engine of the expert system are based on:

a. Environmental Quality Act 1974 (Act 127) (DOE, 2000; DOE, 2001)
b. Occupational Safety Health Act 1994 (Act 514) (MDC, 1996)
c. National Ecotourism Plan Guideline for Malaysia (WWF, 1999)

The primary data collected through questionnaires in the field survey uses the perception of the domestic and foreign tourists and also officers' (persons manning the ecotourism sites) perception in terms of the level of agreement for safety, health and environmental factors on an online Likert Scale as used by Birdir and Pearson (2000) in the Delphi method. Table 2 shows the Alpha Reliability Coefficients result of various independent variables for the pre-testing of the questionnaire.

TABLE 2
Summary of reliability analysis for pre-test

	Independent Variable	Alpha Reliability Coefficients
1	Safety	0.8685
2	Health	0.7709
3	Environment	0.8175
4	Management	0.3108
5	Socio-economic	0.2700
6	Aesthetics	0.0717
7	Management/Socio-economic/ Aesthetics	0.2633

Results show a consistency in the survey questionnaire with a high reliability for safety, health and environmental factors.

Phase 3: Design

The system is designed on a web graphical user interface (GUI) for simplicity and broader networking on an information-sharing platform. The system is designed and developed on a back-end on-line database, which will keep record

of all successful transactions and rate accordingly. *Fig. 1* shows the simplicity of the system design.

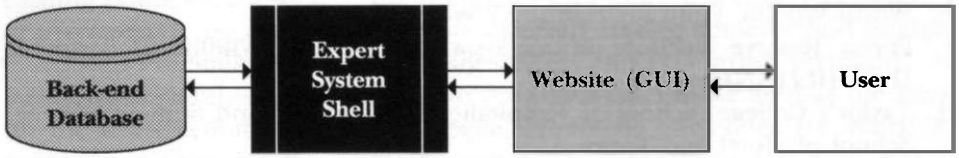

Fig. 1. User Interfacing with database

The system architecture of the rating system is detailed in *Fig. 2*. The home page (main URL) of the site is http://www.ecotoures-upm.net/. The site acts as the GUI for the users (ecotourism operators). The user interface is the vehicle through which the user views and interacts with the system. Microsoft Front Page 2000 is used as the developer interface for the knowledge engineer (the researcher).

The explanation facility subsystem is responsible for providing explanation on the reasoning and acts as a "help" function in the URL: http://~/rating.htm and http://~/help.htm. The algorithm for the working memory, knowledge base and inference engine is written in ASP. In the working memory, users enter information in an ASP generated form (http://~/*.asp). The system matches this information with the knowledge contained in the knowledge base to infer new facts. The facts in the working memory and the knowledge contained in the knowledge base are inferred by the inference engine in an ASP generated handler algorithm (http://~/*handler.asp) to derive new information. It searches the rules for a match between their premises and information contained in the working memory. When the inference engine finds a match, it adds the rule's conclusion to the working memory and continues to scan the rules looking for new matches.

All successful transactions would be updated in the knowledge-based database using external programs such as Microsoft Access 2000. In the rating Website, the database file is stored in he URL: http://~/*.mdb.

All questions are made up of standard HTML and Microsoft Front Page form components integrated with ASP. Generally, each question has a corresponding handler. The functions of a handler are to:

1. extract scores from previous questions
2. convert scores into integers
3. perform calculations on the score, to check if the question was answered adequately and move on to the next question, or to go straight to the summary page and end the session.

The summary page uses scores obtained for each question to tabulate a percentage based on safety, health and environment using different weightage.

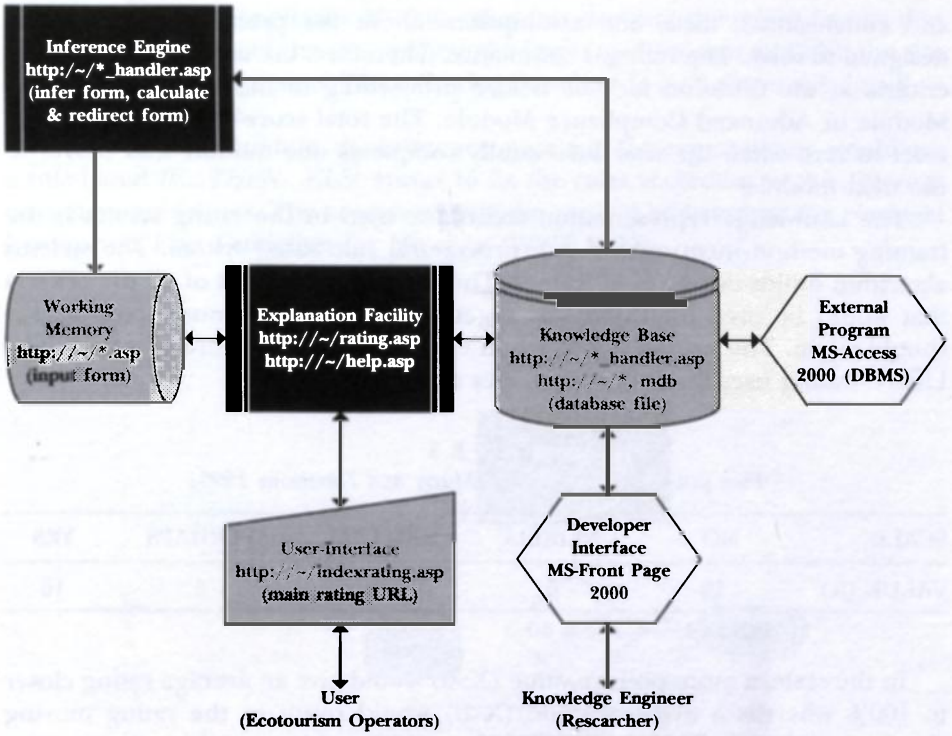

Fig. 2. Rule-based system architecture

The successful Costa Rican hospitality and tourism accreditation model (Font and Buckley 2001) and the Tourism Authority of Thailand rating and ranking system for nature tourism (Services Industries Research Group 1997), are used as the basis for the ecotourES algorithm and model. The general scoring mechanism uses the following equation:

$$\text{Total Score} = \sum_{i=1}^{n} \frac{WiRi}{Wi}$$

Wi = weighted score for given question
Ri = rated score for given question
n = total number of questions

The rating system can be divided into 3 modules, namely the Filtration, Basic Compliance and the Advance Compliance Module. The expert system would ask subsequent questions based on the user input. Therefore, no two users may be asked the same set or the same number of questions. The knowledge is typically represented in the form of *IF... THEN* type rules (premises

and conclusions), facts, and assumptions about the problem the system is designed to solve. The rating is cumulative. Therefore, the user has to fulfill the criteria at the filtration module before proceeding to the Basic Compliance Module or Advanced Compliance Module. The total score for each module is reset to zero when the user successfully completes one module and moves to the next module.

The knowledge representation technique used in the rating system is the framing method incorporated in a procedural rule-based system. The systems algorithm builds two types of frames. The first frame consists of all the criteria that would be used for the rating together with the minimum score a user should attain. The second frame would capture the actual score of a user. The Likert Scaling uses the following values shown in Table 3.

TABLE 3
Five point Likert Scaling *(Murry and Hammons 1995)*

SCALE	NO	UNLIKELY	NEUTRAL	PERHAPS	YES
VALUE (X)	- 10	- 5	0	5	10

In the scale, a more positive value (X>0) would give an average rating closer to 100% whereas a negative value (X<0) would result in the rating moving closer towards 0%. The negative value would be able to reduce the average score computed in each module. Hence, equilibrium can be achieved in the rating.

Each question is weighted by its relevance on a scale of 1 to 3 (normal: low weightage = 1, medium: average weightage = 2 and high: high weightage = 3). Nevertheless, the procedural rule-based algorithm would reduce the total number of questions and options used to answer. This is in contrast to the Costa Rican CST programme where users have to answer all the 153 questions.

In the Filtration Module, users will have to pass the first level of filtration in order to be eligible to use the rating system. The filtration module would encompass the sites' safety, health and environmental compliance according to the ecotourism definition. The acceptable ecotourism definition used for compliance is developed through consultation with the domain expert and secondary data. The filtration level too would filter the eligibility of the user to proceed further in the rating based on the legislative and ecotourism guideline requirements. In the filtration level, a higher weightage is assigned for the ecotourism definition compliance.

The Basic Compliance Module takes into account the macro factors that influence the rating of the site, namely the ecotourism value factors, operator qualification to run the place and permitted/not permitted activities. Here the level of compliance in safety, health, environment and other factors is considered for rating. A higher weightage is assigned for the safety compliance. In this module, users are required to answer all the 5 questions.

The Advance Compliance Module takes into account the micro factors that influence the rating of the site, namely the impacts of tourism and also the site planning and management. The following flowchart (*Fig. 3*) shows the data flow of the rating system.

In the advance module, the inference engine in the expert system would use a rule-based *IF...THEN...ELSE* syntax to fix the rules according to the different ecosystem guidelines. The ecosystem guideline would be based on the National Ecotourism Plan Guideline for Malaysia (WWF 1999).

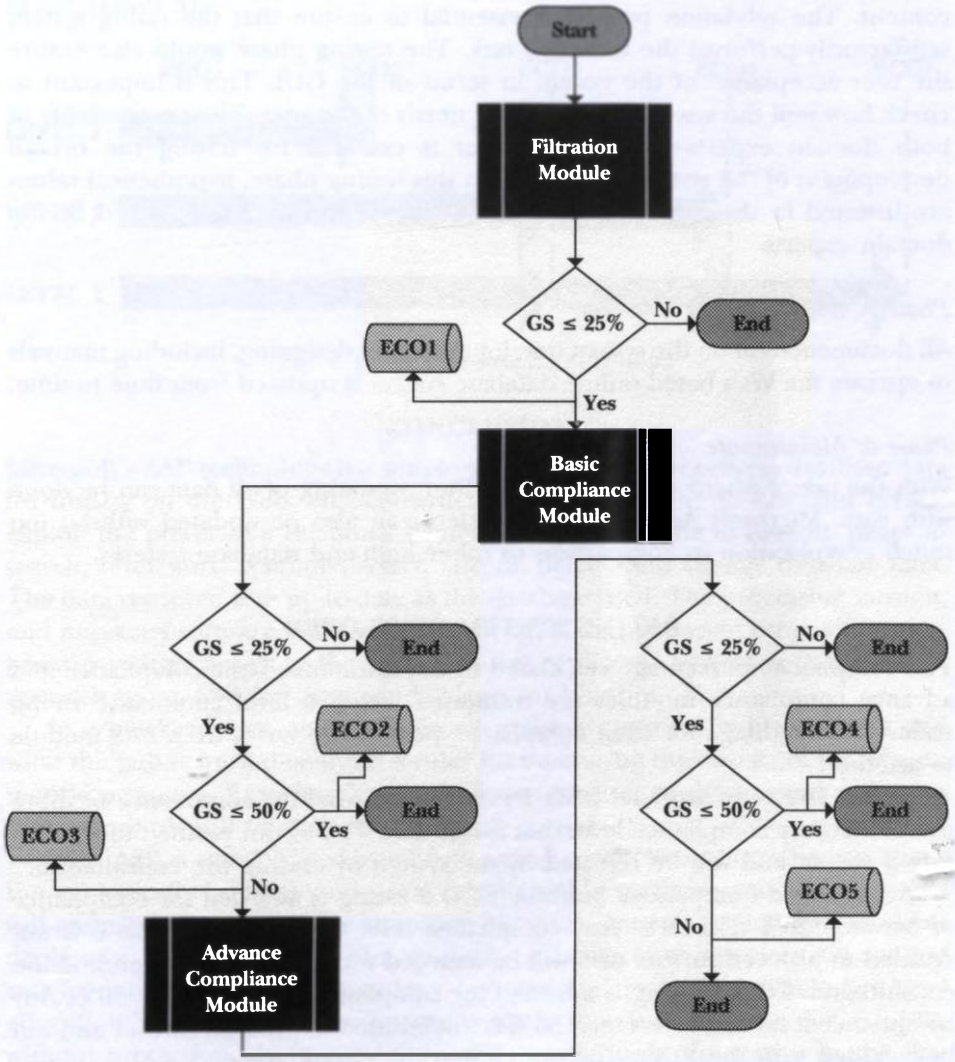

Fig. 3. Data flow of the rating system

Similar to the Basic Module, the level of compliance in safety, health, environment and other factors is considered for rating. In addition, a higher weightage for rating is given in the Advance Module compliance of the National Ecotourism Guidelines.

Phase 4: Testing

The testing phase is an iterative process. New knowledge from the domain expert is added to the system throughout this phase. The major objective of testing is to verify and validate the overall structure of the system and its content. The validation process is essential to ensure that the rating system satisfactorily performs the intended task. The testing phase would also ensure the user acceptance of the system in terms of the GUI. This is important to check how well the system addresses the needs of the user. The acceptability of both domain experts and the end-user is essential for testing the overall development of the system's interface. At this testing phase, hypothetical values are inserted in the ES and the output of the ECO rating is analysed by the domain experts.

Phase 5: Documentation

All documentation on the system development and designing, including manuals to operate the Web based online database system is updated from time to time.

Phase 6: Maintenance

With the use of Microsoft Front Page Editor, updating of all data can be done with ease. Microsoft Access database system can also be updated without too much complication in comparison to other high-end database systems.

RATING SYSTEM DEVELOPED

The compliance percentage calculated in the filtrations, basic compliance and advance compliance modules are translated into a 5 level cumulative rating scale. The weightage for each compliance percentage varies from one module to another.

At the Filtration Module, ECO 1 rating is awarded for compliance of more than 75%. Any compliance level that is less than 75% is not entitled to use this rating system and will be rejected by the system by ending the consultation.

At the Basic Compliance Module, ECO 2 rating is awarded for compliance of between 25% and 50%. Any compliance level that is less than 25% is not entitled to proceed further and will be awarded with ECO 1 before ending the consultation. ECO 3 rating is awarded for compliance of more than 50%. Any compliance level that is less than 50% is not entitled to proceed further and will be awarded with the ECO 2 before ending the consultation.

At the final Advance Compliance Module, ECO 4 rating is awarded for compliance of between 25% and 50%. Any compliance level that is less than 25% is not entitled to proceed further and will be awarded with ECO 3 before

ending the consultation. ECO 5 is the highest rating awarded for compliance of more than 50%. Any compliance level that is less than 50% is not entitled to proceed further and will be awarded with the ECO 4 before ending the consultation.

The rating can be summarised as shown in *Fig. 4*. A successful rating can be accomplished in one of the 3 levels. The user can be awarded the rating from ECO1 gradually to ECO2 (Level 1) before terminating the rating. The user too can be rated from ECO1 to move directly to ECO3 and then gradually to ECO4 (Level 2). In the last type, the user can move directly from ECO1 to ECO3 to ECO4 (Level 3).

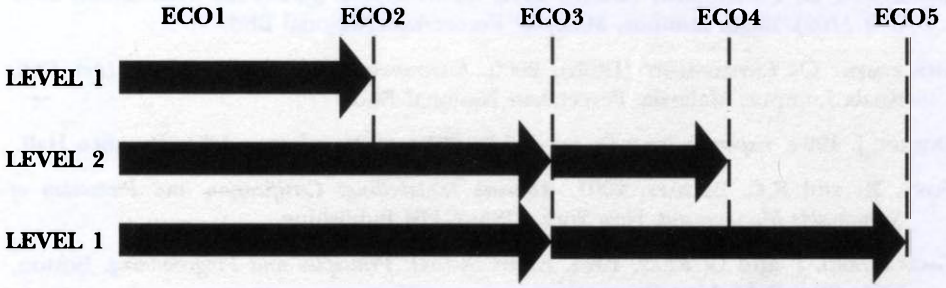

Fig. 4. Cumulative score for ECO rating

CONCLUSION

Microsoft's ASP technology is a simple way to retrieve and process database data for display on the Web. Well-constructed pages consisting of ASP and HTML exploit the power of a relational database. ASP allows one to develop pages to search, filter, sort, combine, select, add to, delete, and change database data. The data retrieved is as up-to-date as the database itself. The processing burden, and necessary software, resides on the server, allowing anyone using almost any browser to have access to the data. This was confirmed by Yerkey (2000) in his research using ASP for dynamic database for libraries.

In a market-driven environment, what the ecotourism industry needs and what the public must demand is a ruler for measuring the impact of tourism on natural resources. Ensuring that nature-based tourism establishes and maintains high standards will be a challenge for all parties.

With ASP, a standardised intelligent rating system like ecotourES can check the ecologically sensitive and economically viable methods and practices that will ensure the survival of the attraction of nature, without having the resources depleted. The management of a sensitive ecosystem in the ecotourism context can in one way protect a country's heritage and make it available for local education and tourism. The environment is the resource base for tourism; without protection, the natural attraction that brought the tourist in the first place will be lost. With the full enforcement and acceptance of a standardised rating system, the possibility of truly sustainable tourism can be a reality.

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Electrochemical Behavior of Polycrystalline Copper in Aqueous Phosphate Buffered Solution During CO_2 Reduction

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ABSTRAK

Proses redoks yang terjadi di permukaan elektrod kuprum dalam larutan tampan fosfat telah dikaji. Hasil kajian menunjukkan bahawa proses pengoksidaan dan penurunan yang terjadi di permukaan kuprum terjadi dengan perlahan dengan proses evolusi hidrogen terjadi pada nilai keupayaan yang lebih negatif dari -1.0 V. Proses evolusi hidrogen ini didapati tidak dipengaruhi oleh tahap keupayaan anodik yang dikenakan. Lapisan filem tipis yang terbentuk di permukaan kuprum mengalami tindak balas penurunan secara proses berbalik-quasi. Dalam larutan yang ditepukan dengan gas CO_2, kehilangan kuprum ke dalam larutan terjadi disebabkan oleh pembentukan spesies terlarut. Proses kehilangan ini meningkat melalui proses pempolaran permukaan elektrod di mana kuprum(I)-karbonil terbentuk dan larut ke dalam larutan. Hasil kajian juga menunjukkan sifat-sifat elektrokimia elektrod kuprum berubah secara dramatik melalui proses pempolaran. Melalui proses ini, kawasan evolusi hidrogen terhalang dengan jayanya disebabkan proses penurunan CO_2 terjadi. Proses penurunan ini meningkat dengan hanya menggunakan elektrod kuprum yang telah dipolarkan pada keupayaan katod yang tinggi untuk jangka masa tertentu.

ABSTRACT

The redox processes occurring on the copper surface in phosphate buffered solution were investigated. The results show that the oxidation and the reduction of copper surface occur in a slow process with hydrogen evolution region proceeding at potential more negative than -1.0 V. It is found that the hydrogen evolution region was not affected by the anodic potential limits. The anodic film forms on the copper surface are reduced possibly in quasi-reversible manner. In CO_2-saturated solution, the loss of copper to the solution increases due to the formation of copper soluble species. This process is further enhanced with the polarization process, where copper(I)-carbonyl forms to the bulk solution. The results also show that the electrochemical behavior of copper electrode is dramatically changed by the polarization process. The hydrogen evolution region is greatly depressed due to the CO_2 reduction process. Consequently, the reduction of CO_2 is not pronounced on the copper surface except for the electrode surface has which been polarized at high cathodic potential for a period of time.

Keywords: Electrochemical, polycrystalline copper, CO_2 reduction

INTRODUCTION

It is a well-known fact that gaseous CO_2 dissolves in water in an amount that depends on a series of equilibrium steps (Aurian-Blajeni 1992). On dissolution, the species formed are: aqueous carbon dioxide, carbonic acids, hydrogen carbonates and carbonates; the quantity of each depends, amongst other factors, on the pH of the solution. Therefore, the need for monitoring this process in aqueous media such as phosphate buffered solution becomes important, as the nature of the reduction products depends on a variety of experimental conditions.

It is clear from the previous report that the control of pH is an important issue when discussing the reduction of CO_2 (Jumat 2001). The loss of CO_2 through the carbonate route is a consequence of pH change. This was due to the very basic conditions appearing across the double layer as a consequence of the onset of hydrogen evolution. This leads to a substantial decrease in the CO_2 available for the reduction process. This result agrees with the general fact that no reduction products are obtained in nitrogen-saturated hydrogen carbonate or carbon dioxide-saturated solution (Jumat and Kalaji 2002) and with the general low faradaic yields observed for the process. However pre-treatment such as the removal of the species that enhance the basic environment of the electrode surface such as "hydroxide" ions may increase the faradaic yields. Polarization of the electrode surface at high cathodic potential is one of the novel ways (Jumat and Kalaji 2002a). In this report, we discuss the results of our study of the processes occurring at and near the surface of Cu electrode in phosphate buffered solution. The use of buffer solution such as phosphate buffer that can maintain the pH of the electrolyte solution, also in the thin layer during the electrochemical reduction is worth trying. Beside its better pH control, phosphate buffers do not exhibit any absorbance that may overlap with the carbonate-hydrogen carbonate system during Substratively Normalized Infrared Fourier Transform Spectroscopy, SNIFTIRS measurements.

MATERIALS AND METHODS

Experimental details in the present study have been reported elsewhere (Jumat and Kalaji 2002). Electrochemically polished copper electrodes used throughout this work were cathodically protected by applying a negative potential immediately after its immersion into degassed phosphate buffered solution (pH 6.8). A reducing potential close to or at the hydrogen evolution potential was applied for few minutes before the cyclic voltammetry measurements in order to remove any oxides or hydroxides from the surface. Nitrogen gas was bubbled through the solution between the experiments and passed over the electrolyte quiescently during experiments. CO_2 was bubbled for 15 min to prepare CO_2-saturated solutions for the electrochemical reduction experiments.

The electrolyte solution at low temperature ~ 5^0C was first degassed with N_2 and then either saturated with CO_2 for 15 min or CO_2-saturated while the admission potential at high negative of -1.5 V was employed as soon as the

electrode was immersed in the solution. The admission potential holding time was varied prior to the spectra measurement. The *in situ* IR spectra measurements were performed in a staircase mode using a fully evacuated FTIR spectrometer (Bruker IFS-113V) fitted with a mercury-cadmium-telluride (MCT) photoconductive detector cooled at 77^{0}K (using liquid nitrogen), p-polarizer and Ge/KBr beam splitter. The spectrometer was fully computer controlled running under OPUS 3.0 software. SNIFTIRS spectra collection was performed with 200 scans at a resolution of 4. The potential either generated by Hi-Tek DT-2101 potentiostat and PP-R1 waveform generator or controlled by an ADC converter interface to a PC that controlled the spectrometer, was stepped by 100 mV during each step. The spectral collection was performed first in the negative direction (from -0.7 V) towards a potential of -1.5 V then towards positive potential of 0.0 V, then back to the initial potential of -1.5 V.

RESULTS AND DISCUSSION

Voltammetry of Copper in Buffered Phosphate Solution

The electrochemical behavior of copper in phosphate solution has been reported elsewhere (Osamu and Hori 1993; Hernandez and Kalaji 1996; Drogowska *et al.* 1992; Ribotta *et al.* 1995; Alhaji and Reda 1996). In general, in N_2-nitrogen saturated buffered phosphate solution (*Fig. 1*) an anodic wave corresponding to the formation of copper oxides and/or hydroxides of Cu(I) and Cu(II) is observed at about -0.12 V during the forward sweep from -1.1 V to 0 V. However there is no clear peak separation between the formation of oxide and hydroxide of Cu(I) and Cu(II) as was observed in the hydrogen carbonate solution (Jumat and Kalaji 2002; Hernandez and Kalaji 1996). On the reverse sweep from 0 V to -1.1 V, it is clear that the reduction of such oxides occurs at about -0.23 V. At low sweep rate, 0.005 V/s, the oxidation and the reduction peaks are much sharper than that at 0.05 V/s. The result shows that the oxidation and the reduction of copper surfaces may be a slow process. Hydrogen evolution reaction, HER commences at potentials more negative than -1.0 V.

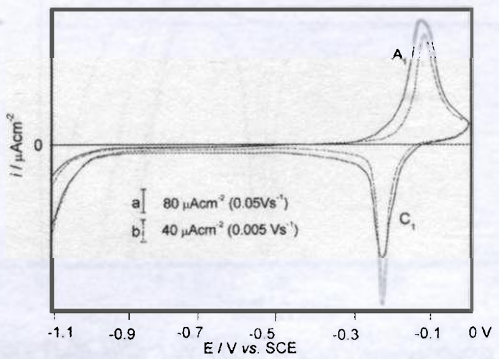

Fig. 1: Voltammogram for copper electrode at (a) v = 0.05 and (b) 0.005 Vs^{-1} in N2 saturated phosphate buffered solution (pH 6.8) at 18°C

The effect of the anodic limit on the shape of the voltammograms is illustrated in *Fig. 2*. The onset of the hydrogen (not shown) was not affected by the positive potential limit. The current profiles in the positive direction (from -0.6 V) show a broad region with only one noticeable current peak, A_1 at -0.04 V, whereas during the negative sweep, one cathodic peak, C_1 at -0.16 to -0.18 V is observed. The broad anodic peak is due to the copper dissolution and anodic film growth (Alhaji and Reda 1996). The position and the charge of anodic current peak A_1 were maintained for several successive runs whereas the structure and the position of the cathodic peak depended on the positive potential limit. As the positive potential limit became more positive, the charge of the cathodic current increased, whereas the peak potentials shifted in the negative direction. The difference in shape between the anodic and cathodic peaks is most likely due to different reaction mechanisms. Therefore the oxidation is most likely to be diffusion controlled and will be discussed in detail elsewhere, whereas the reduction step is kinetically controlled. Such behavior is known in electrochemical system such as the reversible conversion of Pb to $PbSO_4$ in sulphuric acid (Jumat 2001).

The charge density associated with the cathodic wave, Q_{red} (0.82 mC) was always smaller than the one associated with the anodic peak, Q_{ox} (0.95 mC). This indicates a loss of copper species into the solution, possibly through copper species such as CuO or $CuHPO_4$. It is possible but unlikely that the difference in this value could be due to the accumulation of unreduced anodic film on the copper surface. However, since the voltammograms show a good reproducibility on all subsequent anodic sweeps under repetitive cycling conditions with cathodic and anodic peaks separation value of 120 mV, it can be concluded that any anodic film formed on the copper surface was easily reduced, possibly in quasi-reversible manner (Pyun and Park 1986).

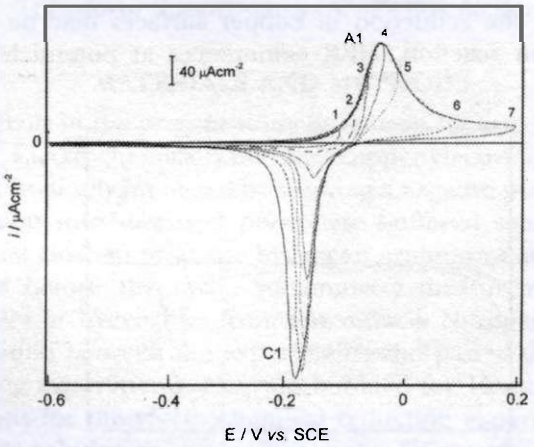

Fig. 2: Voltammogram of copper electrode at ν = 0.005 Vs-1 in buffered phosphate solution (pH 6.8) at 18°C. Potential range from (1) -0.6 to -0.1 V; (2) -0.6 to -0.075 V; (3) -0.6 to -0.05 V; (4) -0.6 to 0.025 V; (5) -0.6 to 0 V; (6) -0.6 to 0.1 V and (7) -0.6 to 0.2 V

Voltammetry of Copper in CO_2 Saturated Phosphate Buffer Solution

The introduction of CO_2 into phosphate buffer solution has an impact on HER as well as the redox behavior of copper. *Fig. 3* shows the difference in voltammetric behavior between N_2-saturated and CO_2-saturated solution (*Figs. 3a* and *3b* respectively). It is interesting to note that HER occurs at very similar potential in the two solutions, which is in contrast to hydrogen carbonate solutions. This is due to the buffering effect of the phosphate solution, in which the pH drops by only 0.6 units upon saturation with CO_2. The oxidation of the Cu surface and the corresponding reduction of solution species and deposits is similar to that observed in hydrogen carbonate solutions; however, an asymmetry was observed here between Q_{red} and Q_{ox}; indication of a loss of soluble species to the bulk solution.

The average of the Q_{red}/Q_{ox} efficiency is about 70% (*Fig. 3b*). It is possible and likely that the difference in this value could be due to the accumulation of unreduced anodic film on the copper surface, which is related to the formation of species that are related to the oxidation of copper species at anodic potential region. The formation of oxidized copper film (oxides and/or hydroxides) and soluble copper species result from the interaction of reduced-CO_2 species such as CO with oxidized copper also believed to occur at this potential. It is interesting to highlight that once the electrode was first polarized at negative potential, E_{pol} of -1.5 V for 15 min prior to the voltammogram measurement, the average of the Q_{red}/Q_{ox} efficiency decreases dramatically to about 30% (*Fig. 3c*). It indicates that the formation of soluble copper species increased at anodic potential dramatically due to the polarization of the electrode surfaces at high negative potential.

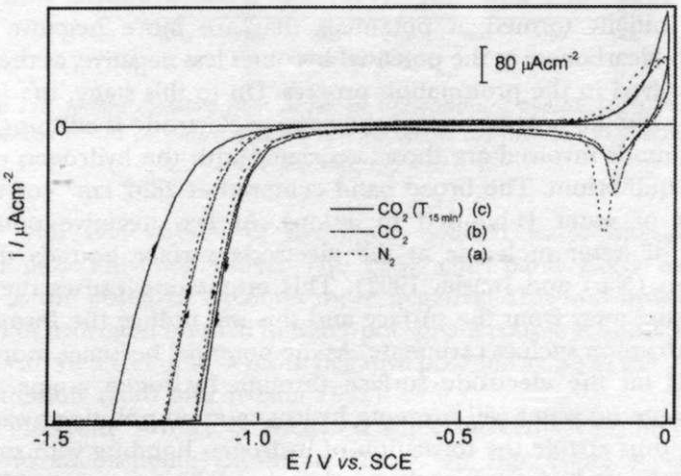

Fig. 3: Voltammogram for copper electrode at v = 0.05 Vs^{-1} in (a) N_2 saturated phosphate buffered solution, (b) CO_2 saturated without and (c) with polarization at polarization potential of -1.5 V for 15 min

***In situ* FTIR**

SNIFTIR Spectra of N2-saturated Buffered Phosphate Solution

The use of phosphate buffers has some advantages when compared with bicarbonate solution in that it allows a better pH control and reduces to a certain extent the interference from the hydrogen carbonate ions. However, the strong absorbance exhibits will mask the spectral range from 1300-900 cm^{-1}, by phosphate buffers. It is important to be able to distinguish the background spectrum of the electrolyte from that of the electroactive species. Therefore the SNIFTIR spectra of the phosphate solution in responses to potential changes were recorded. *Fig. 4* shows the SNIFTIR spectra of the phosphate buffered solution, which has been saturated with pure N_2 gas. The positive bands at 1160 cm^{-1} (ν_{as}-PO_2), 1076 cm^{-1} (ν_s-PO_2) and 943 cm^{-1} (ν_{as}-$P(OH)_2$) correspond to the depletion of monobasic phosphate, $H_2PO_4^-$ whereas negative bands at 1061 cm^{-1} (ν_s-PO_2) and 991 cm^{-1} (ν_s-$P(OH)_2$) correspond to the formation of dibasic phosphate, HPO_4^{2-} as the potential becomes more negative (Chapman and Thirlwell 1964, Weber and Nart 1996). The main negative band at 1010 cm^{-1} (ν-PO_3) can be assigned to the formation of tribasic phosphate, PO_4^{3-} as HPO_4^{2-} is deprotonated, as the potential is made more negative. Consequently, a broad negative band at 2720 cm^{-1} appears as the potential becomes more negative. It is attributed to the OH stretching for the hydrogen bonding that forms between H_2O molecule and PO_4^{3-} (Weber and Nart 1996).

Fig. 5 shows SNIFTIR spectra of copper in CO_2-saturated phosphate buffered solution as the initial potential at -1.5 V is stepped towards positive values as shown in scheme A (*Fig. 6*). The spectra are normalized relative to E_{ref} -0.6 V. The spectra obtained are similar to those obtained in CO_2-saturated hydrogen carbonate solution, except for the bands due to the phosphate species. HCO_3^- (1356 and 1302 cm^{-1}) and CO_3^{2-} (1401 cm^{-1}) bands appear with potential. Carbonate initially formed at potentials that are more negative and then converted to bicarbonate as the potential becomes less negative, as the carbonate ions are involved in the protonation process. Up to this stage, the fate of CO_2 during the cathodic polarization of the copper electrode is still unclear as the main compounds involved are those associated with the hydrogen carbonate/ carbonate equilibrium. The broad band centered at 2852 cm^{-1} corresponds to OH stretch of water H-bonded to anions. At less negative potential, the orientation of water molecule at the electrode surface bounds through an oxygen atom (Nart and Iwasita 1992). This orientation causes the hydrogen atoms pointing away from the surface and this will induce the formation of H bonding with anion such as carbonate. As the potential becomes more negative, water bonds on the electrode surface through hydrogen atoms. An excess negative charge on water will promote hydrogen atom pointing away from the surface and thus enable the formation of hydrogen bonding with surrounding anions containing hydrogen such as phosphate. The band at 2756 cm^{-1} is consistent with the band observed for OH stretch of hydrogen bond of water-phosphate couple, as compared to that spectra in N_2-saturated solution where a broad band centered at 2720 cm^{-1} as shown by insert figure in *Fig. 4* which is

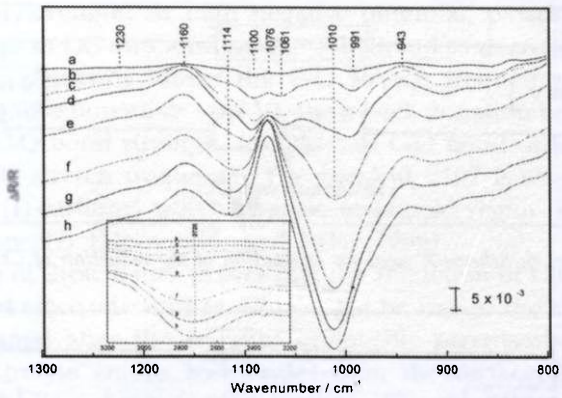

Fig. 4: SNIFTIR spectra obtained from N -saturated phosphate buffered solution as the potential was carried in staircase mode. Spectra shown are from (a) -0.8 V to (h) -1.5 V in 0.1 V per step. E_{ref} = -0.7 V

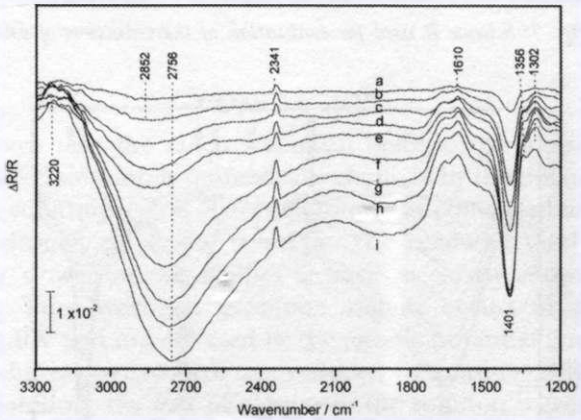

Fig. 5: SNIFTIR spectra obtained from cooper in CO_2 -saturated phosphate buffered solution as the potential was varied in staircase mode. Spectra shown are from (a) -0.7 V to (i) -1.5 V in 0.1 V per step. E_{ref} = -0.6 V

associated with OH stretch of hydrogen bonded water-phosphate couple (Chapman and Thirlwell 1964). The 2852 cm^{-1} band shifts toward lower frequency as the potential becomes more negative. This may indicate that the OH stretch of hydrogen bonded in water-phosphate couple is more predominant than water-carbonate couple at more negative potentials due to the predominant water orientation (Nart and Iwasita 1992).

As the electrode surface is polarized while bubbling CO_2 in the solution according to the scheme shown in *Fig. 7*, an interesting electrochemical behavior (*Fig. 3c*) and SNIFTIR spectra were observed. As shown in *Fig. 8*, several new bands were observed at 1881, 1955 and 2107 cm^{-1}. The band at 1881 cm^{-1} (at -1.4 V) shifts to 1955 cm^{-1} (at -0.2 V), as the potential is made more

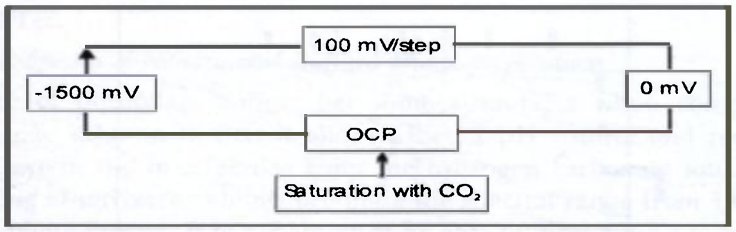

Fig. 6: Scheme A used for evaluation of the reduction of CO_2

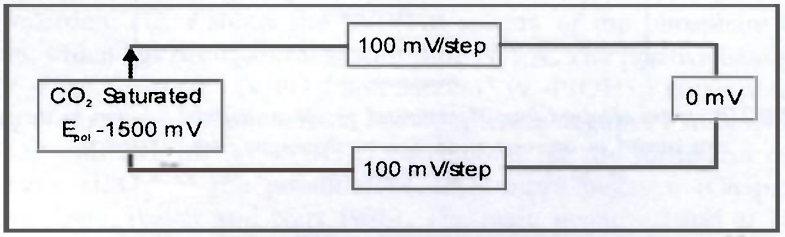

Fig. 7: Scheme B used for evaluation of the reduction of CO_2

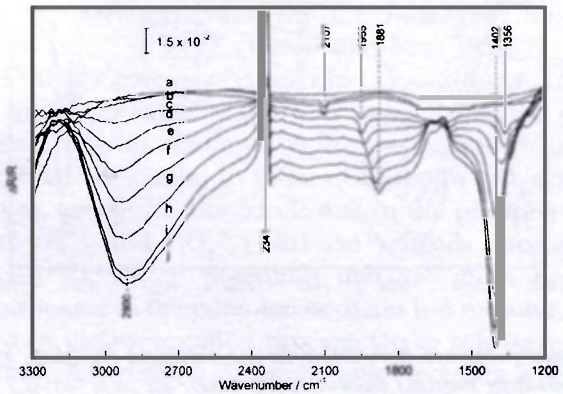

Fig. 8: SNIFTIR spectra obtained from continuous CO_2-saturated buffered phosphate solution as the cooper was polarized at -1.4 V for 15 minutes at 0°C. Spectra shown are from (a) -0.3 V, (b) -0.1 V of reverse sweep, (c) 0 V to (j) -1.4 V of forward sweep in 0.2 V per step. E_{ref} = 0.4 V of reverse sweep

positive. This shifting band proves that the band is associated with adsorbed species (Dovydov 1990; Nakamoto 1986). As reported earlier the band at 1881 cm^{-1} is assigned to adsorb bridge-bonded CO (Jumat and Kalaji 2002). A shift in band position for adsorbed CO has been rationalized by the p-back donation process, which is well documented (Blyholder 1964). The shift to higher frequency as the potential is made more positive indicates an increase in the

C-O bond order/strength. At high negative potential, p-back donation from copper dp orbital to CO antibonding $2\pi^*$ is believed to decrease the C-O bond order which consequently lowers the C-O stretch frequency. On the other hand, at less negative potentials (-0.2 V), the π-back donation becomes less, thus increasing the C-O bond strength. Increases in C-O bond order will therefore increase the C-O stretch frequency. The band at 2107 is assigned to soluble species, copper(I)-carbonyl which appears at anodic region where copper is oxidized to copper(I) (Hernandez and Kalaji 1996).

The existing of these bands proves that the reduction of Co_2 can only occur on the polarized electrode surface. This might be due to the electrode surface turn to be cleaned after the removal of specific pre-adsorbed ions such as hydroxide, electrolyte anions and oxides from the surface, thus leaving the clean surface to allow the reduction process. The electrochemical properties change on the copper surface is clearly shown in *Fig. 3c* where the hydrogen evolution region is depressed and shifted toward more negative potential, implying that some other process occurred in this region. This is consistent with the appearance of new bands of adsorbed CO and copper(I)-carbonyl in the same experimental condition.

CONCLUSION

It is clearly shown that the electrochemical behavior of copper electrode in phosphate buffered solution is dramatically changed by the polarization process. The hydrogen evolution region is depressed greatly compared to what has been reported in hydrogen carbonate solution. The oxidation and the reduction processes occur slowly on the copper surface as clearly shown by the cyclic voltammogram. The hydrogen evolution region occurs at potential more negative than -1.0 V and not affected by the anodic potential limits. The anodic film forms on the copper surface are reduced in quasi-reversible manner. In CO_2-saturated solution, the loss of copper to the solution increases due to the formation of copper soluble species. The rate of copper dissolution to the solution is enhanced through the polarization process, where copper(I)-carbonyl forms at anodic potentials. The reduction of CO_2 will not proceed on the copper surface unless the electrode surface has been polarized at high cathodic potential for a period of time. This might be due to the competing process between the CO_2 reduction and the specific pre-adsorption of anions processes occurring on the electrode surface.

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The manuscript, including footnotes, tables, and captions for illustrations, should be typewritten double spaced on paper 210 x 297 mm in size, with margins of 40 mm on all sides. Three clear copies are required. Typing should be on one side of the paper only. Each page of the manuscript should be numbered, beginning with the title page.

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Up to a maximum of ten keywords are required and they should be placed directly below the abstract.

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